Mucosal endocrine cell micronests and single endocrine cells following neo-adjuvant therapy for adenocarcinoma of the distal oesophagus and oesophagogastric junction

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J Clin Pathol 2007;60:1284–1289. doi: 10.1136/jcp.2007.047449

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Accepted 9 February 2007

Aims: To determine the frequency of endocrine cell micronests (ECM) and single endocrine cells (SEC) within the glandular mucosa of the distal oesophagus and oesophagogastric junction (OGJ) following neo-adjuvant therapy for adenocarcinoma.

Methods: The resection specimens from 11 patients with adenocarcinoma of the distal oesophagus or OGJ who had undergone preoperative chemotherapy or chemoradiotherapy (CRT) were reviewed and stained immunohistochemically for cytokeratin and chromogranin. The presence of ECM and/or SEC within the mucosa adjacent to the tumour was noted, and the results correlated with the extent of tumour regression. The corresponding pretreatment endoscopic biopsy specimens were reviewed in 6 cases, and the results were also compared to 10 tumour resections from patients with no history of neo-adjuvant treatment.

Results: ECM and/or SEC were identified in 8/11 resection specimens after chemotherapy or CRT. The endocrine cells were typically located within the deep lamina propria or muscularis mucosae and were associated with varying degrees of glandular atrophy and inflammation. The appearances were most consistent with endocrine cell preservation (pseudo-hyperplasia) following treatment. Isolated endocrine elements were not seen in the pretreatment biopsy specimens, while rare SEC without ECM were identified in only 2/10 control resection specimens.

Conclusions: Endocrine cell pseudo-hyperplasia may be seen within atrophic glandular mucosa following neo-adjuvant therapy of distal oesophageal/OGJ adenocarcinomas. The changes are analogous to those seen in chronic atrophic gastritis and should not be misinterpreted as those of residual tumour.

Adenocarcinoma of the distal oesophagus and the oesophagogastric junction (OGJ) is now the most common type of oesophageal cancer and appears to be increasing in incidence.^{1–3} Although surveillance of patients with Barrett's oesophagus has led to early diagnosis in some cases, many patients still present with relatively advanced disease and the overall prognosis is poor. The management of patients with potentially curable disease is dependent on the tumour stage and while superficial tumours may be amenable to local ablation, most patients require distal oesophagectomy and proximal gastrectomy. Recently, chemotherapy and/or radiotherapy (CRT) have been introduced prior to surgery with the intention of improving loco-regional control by reducing tumour volume and eradicating nodal metastases ("down-staging"). While there is no clear evidence that neo-adjuvant CRT improves overall survival,^{1,2} patients with complete pathological remission at the time of resection appear to have a better prognosis.^{3–7}

The accurate assessment of tumour staging and the identification of any treatment-related effects depend on the detailed histological examination of resection specimens. Therefore it is self-evident that pathologists need to be aware of the morphological changes that may occur in the oesophagus or stomach following neo-adjuvant therapy. Complete microscopic regression of tumour has been documented in 10–30% cases, while the remaining specimens show variable degrees of persistent neoplasia.^{2–5} Characteristic treatment induced changes within the residual tumour include attenuation of glands, increased cytological atypia, degenerative nuclear changes and decreased mitotic activity.⁸ Cytoplasmic vacuolation and eosinophilia have been described in rectal adenocarcinomas after therapy, and the latter appearances may reflect an

endocrine or oncocytic phenotype.^{9–10} Stromal changes that commonly follow treatment include fibrosis, dystrophic calcification, atypical fibroblasts and vascular obliteration. Mucin pools that are acellular or include only scant residual neoplastic cells are reported in 11% of Barrett's oesophagus-related adenocarcinomas, and this feature is more common in tumours with mucinous or signet ring cell morphology prior to therapy.¹¹ The relative proportions of the reactive fibro-inflammatory stroma and the residual neoplastic elements have been used to grade the effects of CRT in oesophageal adenocarcinoma,^{5,7,8,12} and similar grading systems have been applied in tumours of the stomach¹³ and rectum.^{14–16} Recently, one such grading scheme was shown to have excellent inter-observer agreement.¹⁷

While changes in the neoplastic cells and the stroma following treatment are relatively well documented, few studies have detailed the appearance of the non-neoplastic mucosa other than the documentation of associated ulceration, Barrett's oesophagus or dysplasia. However focal glandular atrophy, epithelial attenuation and apoptosis have been described within the specialised gastric mucosa,⁸ and Brien and colleagues noted that radiation induced atypia within benign glands can mimic dysplasia or even residual carcinoma.¹⁸ Similar epithelial changes together with crypt architectural distortion and fibrosis within the lamina propria and submucosa have been described in treated rectal tumours.^{19,20} We recently observed prominent clusters of endocrine cells (endocrine cell micronests, ECM) and isolated endocrine cells within the non-neoplastic mucosa adjacent to areas of

Abbreviations: CRT, chemoradiotherapy; ECM, endocrine cell micronests; OGJ, oesophagogastric junction; SEC, single endocrine cells

ulceration and tumour regression in oesophagogastric resection specimens following neo-adjuvant therapy. Since, to our knowledge, ECM have not been described in this clinical setting, we examined a series of resection specimens from patients who had undergone preoperative chemotherapy or CRT and compared the results with corresponding preoperative biopsy specimens, and with resection specimens from control cases in which there was no history of prior treatment.

MATERIALS AND METHODS

Twenty-one oesophagogastric resection specimens for adenocarcinoma of the distal oesophagus or the OGJ performed between 2001 and 2006 were retrieved from the histopathology files of PathWest, Sir Charles Gairdner Hospital and SJOG Pathology, Perth, Western Australia. Eleven patients had received chemotherapy ($n = 2$) or combined chemoradiotherapy (CRT, $n = 9$) prior to surgery. The chemotherapy regime comprised 5-fluorouracil and cisplatin given over 5 cycles, while the radiotherapy consisted of 30–45 Gy given in 15–25 fractions. Endoscopic biopsy specimens of tumour taken prior to therapy were available for review in 6 of the 11 treated cases; the histopathology reports were available in 4 additional cases. The remaining 10 patients underwent surgery for adenocarcinoma during the same period but had not received neo-adjuvant treatment: these control cases were matched as closely as possible for age, sex and tumour site. None of the patients had a history of pernicious anaemia, atrophic gastritis or biopsy proven *Helicobacter* infection.

The resection specimens were assessed for the grade and extent of tumour, the presence of lymph node metastasis, and the presence of concurrent Barrett's oesophagus (defined as intestinal type goblet cells within glandular mucosa abutting the oesophagogastric junction). In some cases a diagnosis of Barrett's oesophagus was established only in preoperative endoscopic biopsy specimens. In those cases that had been subject to preoperative treatment, the extent of tumour regression was assessed using a semi-quantitative grading system based on the relative proportion of reactive fibro-inflammatory stroma and of residual tumour as follows: tumour regression grade 1, complete regression; grade 2, rare residual cancer cells; grade 3, increased tumour cells but predominant reactive stroma; grade 4, residual tumour predominates over stroma; grade 5, no regression.¹²

The non-neoplastic mucosa immediately adjacent to the tumour (or, if appropriate, adjacent to areas of tumour regression) was examined for evidence of ECM and isolated endocrine cells. The assessment was made on both conventional H&E stained sections and on slides immunostained for cytokeratin (AE1/AE3, dilution 1:100) and chromogranin A (dilution 1:100) using primary antibodies obtained from Dako (NSW, Australia). Immunohistochemistry was performed using

an auto-immunostainer (Autostainer Plus, Dako) according to the manufacturer's instructions. Appropriate positive and negative controls (primary antibody omitted) were used with each staining batch, and all sections included internal positive controls of normal glands and endocrine cells. Endocrine cell micronests were characterised by clusters of 5–15 cells, usually arranged in rounded aggregates or short cords, situated within the lamina propria or less commonly within the muscularis mucosae. The cells had uniform, round to ovoid nuclei, evenly dispersed chromatin pattern and relatively scant cytoplasm. Some cells had eosinophilic cytoplasmic granules. An attempt was made to semi-quantify the endocrine cell population based on the number of chromogranin immunoreactive cells/mm length of muscularis mucosae. However it was not possible to obtain reliable results due to the very focal nature of the ECM, the variable thickness of the associated mucosa (which influenced the number of residual intra-glandular endocrine cells), and differences between the types of mucosa between the treated and control cases.

The nature of the adjacent glandular mucosa (gastric body, non-specialised gastric/cardiac or intestinal type), the degree of glandular atrophy and the presence of associated inflammatory infiltration were noted.

The study was approved by the institutional ethics committee.

RESULTS

Tables 1 and 2 summarise the clinical and pathological findings of the cases that underwent neo-adjuvant therapy. There were 9 men and 2 women; mean age was 62.4 years (range 53–74 years). Eight patients were considered to have distal oesophageal adenocarcinomas; four of these cases had Barrett's oesophagus in their resection specimens or preoperative biopsies. The tumours appeared localised to the OGJ in three patients, none of whom had confirmed Barrett's oesophagus. The 10 control cases comprised 7 men and 3 women; mean age was 63.7 years (range 52–79 years). Seven tumours were of distal oesophageal origin, five of which were associated with Barrett's oesophagus, while three cases appeared to arise at the OGJ.

All of the oesophagogastric resection specimens following neo-adjuvant therapy showed evidence of treatment effect including complete microscopic regression of tumour in 5 cases and partial regression in 6 cases (table 2). Endocrine cell micronests were identified in 6/11 resection specimens following chemotherapy or CRT while single endocrine cells not associated with glands were seen in 5 cases, 3 of which also included ECM (table 2). No isolated endocrine elements were present in the 6 reviewed preoperative biopsy specimens. Rare SEC were present in 2/10 control cases but ECM were not identified.

Table 1 Summary of clinical and pretreatment pathological findings in 11 patients who had chemotherapy or chemoradiotherapy

Patient no.	Age/sex	Tumour site	Biopsy findings	Barrett's oesophagus	Treatment
1	67 M	DO	PD adenocarcinoma	Yes	CRT
2	60 M	DO	PD adenocarcinoma with signet ring cells	Yes	Chemotherapy
3	74 M	DO	N/A	Yes	CRT
4	73 M	DO	MD adenocarcinoma	No	CRT
5	54 F	DO	MD adenocarcinoma	No	CRT
6	59 M	DO	MD adenocarcinoma	No	CRT
7	56 M	OGJ	PD adenocarcinoma with signet ring cells	No	CRT
8	68 M	OGJ	PD adenocarcinoma with signet ring cells	No	CRT
9	65 M	OGJ	Signet ring cell adenocarcinoma	No	CRT
10	57 M	DO	PD adenocarcinoma	Yes	CRT
11	53 F	DO	PD adenocarcinoma	No	Chemotherapy

DO, distal oesophagus; OGJ, oesophagogastric junction; N/A, not available; MD, moderately differentiated; PD, poorly differentiated; CRT, chemoradiotherapy.

Table 2 Summary of pathology finding in resection specimens following neo-adjuvant therapy

Patient no.	Resection specimen findings	LN positive/ total examined	Tumour regression grade ¹²	Mucosal endocrine cells
1	No residual mural tumour; mucin pools	1/7	Complete (grade 1)	SEC
2	No residual mural tumour; focal high-grade dysplasia; mucin pools	0/7	Complete (grade 1)	No
3	PD adenocarcinoma extending to CRM.	1/8	Partial <50% (grade 4)	ECM
4	MD adenocarcinoma extending to CRM	–	Partial >50% (grade 3)	No
5	No residual mural tumour	1/8	Complete (grade 1)	ECM and SEC
6	Focal tumour in muscularis propria; CMV noted	2/15	Partial >50% (grade 2)	SEC
7	No residual mural tumour	3/3	Complete (grade 1)	No
8	No residual mural tumour; mucin pools	0/4	Complete (grade 1)	ECM
9	PD adenocarcinoma extending to CRM	0/8	Partial <50% (grade 4)	ECM
10	PD adenocarcinoma extending to CRM	4/14	Partial <50% (grade 4)	ECM and SEC
11	PD adenocarcinoma extending to CRM	1/12	Partial >50% (grade 3)	ECM and SEC

LN, lymph nodes; MD, moderately differentiated; PD, poorly differentiated; CRM, circumferential (radial) resection margin; CMV, cytomegalovirus; SEC, single endocrine cells; ECM, endocrine cell micronests.

The ECM were readily apparent on H&E stained sections in four of the post-treatment resection specimens, but less conspicuous in the other two cases where they were identified clearly using only immunohistochemistry for cytokeratin and chromogranin. Similarly, SEC were mainly observed in the immunostained slides where their scattered distribution in the lamina propria deep to intact glands imparted a “raining down” appearance on low magnification (figs 1 and 2). All of the endocrine cell foci were associated with moderate to marked glandular loss or attenuation, and a variable inflammatory infiltrate comprising lymphocytes, plasma cells and eosinophils. The persisting glands generally exhibited a non-specialised gastric mucinous (cardiac) appearance but gastric body type glands were also present (figs 2 and 3). The loss of glands was most marked in the mid to deep mucosa, but in the most severely affected cases only rare surviving glands persisted between the muscularis mucosae and the surface epithelium (fig 4). In such cases, endocrine elements were often evident within the muscularis mucosae which appeared thickened and disorganised (fig 4). Focal cellular atypia consistent with the prior therapy was present in most cases, and one specimen showed occasional cytomegalovirus (CMV) inclusions. Intestinal differentiation was not identified adjacent to ECM. The expression of chromogranin within residual tumour was not examined in detail in this study, but there was no significant immunoreactivity within the neoplastic cells of four cases examined (fig 5).

There was no clear relationship between the presence of endocrine elements and the tumour site, the extent of treatment-related regression, or the presence of associated Barrett's mucosa (table 2), although too few cases were examined to draw definite conclusions. Similarly, there was no clear difference between the cases treated by CRT or those receiving only neo-adjuvant chemotherapy since endocrine elements were identified in both groups. There was no apparent

endocrine cell proliferation within the mucosa distant from the therapeutic field such as the gastric resection margin. All cases from both the treatment and control groups showed focal chronic inflammation of mild to moderate degree within the gastric and/or Barrett's type mucosa. None of the cases showed active *Helicobacter* infection and there were no histological findings to suggest an autoimmune gastritis.

DISCUSSION

Preoperative CRT is used increasingly in the management of patients with gastrointestinal adenocarcinomas and therefore it is important that pathologists are familiar with the morphological changes that can occur as a consequence of treatment. Characteristic changes in both the tumour and the stroma have been described but the non-neoplastic mucosa has received less attention. In this study we have documented an alteration in the benign mucosa adjacent to residual tumour (or areas of apparent tumour regression), namely prominence of isolated endocrine cells and ECM. These changes were not observed in the glandular mucosa distant from the treated tumours or in corresponding pre-treatment endoscopic biopsy specimens. While rare single endocrine cells were seen in two control cases, ECM were not identified. Therefore it appears most likely that the changes were related to the neo-adjuvant therapy.

There are two possible explanations for the finding we have described. Firstly, the endocrine cells could be derived from the tumour, or alternatively they could be non-neoplastic representing normal or reactive mucosal elements. The first hypothesis is tenable since it is well documented that adenocarcinomas of the oesophagus and stomach, like tumours at many other sites, show focal endocrine cell differentiation which can be demonstrated using immunohistochemical stains.^{21–23} Endocrine cells are also present in Barrett's oesophagus including areas of dysplasia.^{23–24} While cells showing an endocrine phenotype usually comprise a minor

Figure 1 (A) There is a small focus of residual poorly differentiated carcinoma within the fibrotic submucosa (right). (B) Immunohistochemistry for cytokeratin highlights the tumour and also shows scattered isolated endocrine cells within the deep lamina propria (arrows).

Figure 2 (A) Gastric body mucosa showing focal glandular atrophy and inflammation. Mucin pools are seen within the superficial submucosa but residual tumour is not identified. (B) Immunohistochemistry for cytokeratin shows a "raining down" appearance due to isolated endocrine elements within the deep lamina propria. (C) At higher magnification endocrine cell micronests and single cells are apparent.

component of adenocarcinomas, it is conceivable that neoplastic endocrine cells could be relatively resistant to the effects of treatment and therefore show increased prominence following CRT. In accord with this possibility, a recent study by Wang and colleagues demonstrated neuroendocrine differentiation in 52% of treated oesophageal and OGJ adenocarcinomas after CRT.²⁵

The proportion of tumour cells immunoreactive for chromogranin and/or synaptophysin was also increased significantly in comparison with the corresponding pretreatment endoscopic biopsy specimens. Conversely, it is possible that treatment could induce or increase endocrine differentiation within some adenocarcinomas. Shia *et al* demonstrated endocrine morphology and chromogranin immunoreactivity in 68% of rectal adenocarcinomas after preoperative CRT compared with 18% of untreated controls.⁹ The endocrine component accounted for more than 20% of the persisting tumour in almost half of the cases they examined. The authors considered that the endocrine phenotype was most likely induced by the CRT rather than caused by persistence of pre-existing neoplastic endocrine elements. Recently, Hornick and colleagues illustrated nests of cells with neuroendocrine phenotype in three oesophageal adenocarcinomas after neo-adjuvant therapy.¹¹ However, the authors did not comment on the distribution of the cells or the significance and cause of this finding.

Alternatively, the endocrine cells could represent non-neoplastic cellular elements, and we believe this to be the more likely explanation for the changes that we have described. The ECM and isolated endocrine cells were cytologically uniform in contrast to the atypical endocrine elements reported by Shia *et al*, and by Wang and colleagues.^{9, 25} More importantly, the ECM were confined to the mucosa and mainly distributed within the deep lamina propria as expected for residual normal endocrine elements. Furthermore, although we did not formally investigate the endocrine phenotype of the tumours in our resection specimens, significant chromogranin immunoreactivity was not observed in four cases in which residual tumour was present adjacent to ECM. Endocrine cell hyperplasia within the mucosa adjacent to the tumour might explain our findings since this feature has been described in association with gastric adenocarcinoma.^{22–26} However the absence of such changes in the pretreatment biopsy specimens or in the mucosa of the control cases makes this hypothesis less likely. The morphological changes in our cases were reminiscent of those seen in chronic atrophic (autoimmune) gastritis in which the fundic endocrine cells show a spectrum of changes from simple clustering to overt carcinoid tumours. In this context, Iwai and colleagues distinguished passive clustering, which they designated "atrophic endocrine cell micronests", from hyperplasia, mainly on the basis of the number of endocrine cells present.²⁷ Atrophic nests typically had less than 10 endocrine cells in each group, similar to most of the lesions we identified. Itsuno and colleagues regarded ECM as neoplastic if they were more than 0.1 mm diameter, exhibited nuclear atypia or carcinoid architecture, or showed infiltration of the muscularis mucosae or deeper gastric wall structures.²⁸ The endocrine cell clusters in our cases met only the last of these criteria in some instances, since occasional cell groups were admixed with muscle fibres of the thickened and disorganised muscularis mucosae. However, we do not believe that this latter feature represented genuine

Figure 3 (A) Gastric body type mucosa with residual glands showing attenuated epithelium and focal atypia. Occasional endocrine cell micronests can be identified (arrow). (B) Immunohistochemistry for chromogranin highlights the endocrine cell micronests (long arrows) and shows single endocrine cells (short arrows).

Figure 4 (A) The mucosa is markedly attenuated including only sparse mucinous glands. Endocrine cell nests are evident within the fibrotic lamina propria and thickened muscularis mucosae. (B) Immunohistochemistry for chromogranin highlights the prominent endocrine elements.

invasion since submucosal involvement was not identified. Solcia and colleagues distinguished “pseudo-hyperplasia” of endocrine cells from genuine hyperplasia or neoplasia by the former’s lack of association with diffuse or linear forms of endocrine hyperplasia, a feature not identified in our cases.²⁹ Thus, by analogy to atrophic gastritis, we believe that the ECM observed in our cases most likely represented residual normal mucosal elements made relatively conspicuous by the associated glandular loss (passive clustering, atrophic ECM or pseudo-hyperplasia).

The presence of mucosal ECM in treated gastro-oesophageal adenocarcinoma is not likely to present diagnostic difficulty in most cases. The distinction from residual adenocarcinoma will seldom be problematic since the persisting tumours usually retain a glandular pattern and the cells typically exhibit marked nuclear atypia with conspicuous eosinophilic or vacuolated cytoplasm. However, occasionally carcinomas (including four cases in our series) may consist partly or predominantly of poorly differentiated single cells or small clusters of cells lacking distinct glandular architecture and these can more closely mimic endocrine cells. It is also pertinent that the neoplastic endocrine cells illustrated by Hornick *et al* following neo-adjuvant treatment of oesophageal adenocarcinoma appeared to be composed of relatively small, uniform cells.¹¹ In problematic cases the distribution of the cells is of great value since benign endocrine elements are restricted to the mucosa, although the occasional intermingling of endocrine cells within the thickened muscularis mucosa should be noted. Immunohistochemical studies may also prove helpful since most carcinomas will be negative, or only focally positive, for neuroendocrine markers.

Figure 5 Immunohistochemistry for chromogranin shows no staining within intra-mucosal adenocarcinoma (lower field). There is immunoreactivity within an entrapped endocrine cell micronest (arrow) and in adjacent benign mucosal glands (upper field).

Take-home messages

- Endocrine cell nests and single endocrine cells may be seen in the glandular mucosa of the distal oesophagus and oesophago-gastric junction following neo-adjuvant therapy for adenocarcinoma.
- The changes most likely represent persistence of normal endocrine elements made relatively conspicuous by the associated glandular atrophy (pseudo-hyperplasia).
- Prior chemoradiotherapy should be considered in the differential diagnosis of endocrine cell pseudo-hyperplasia, and the changes should not be misinterpreted as representing residual neoplasia.

In summary, we describe prominent endocrine cell clusters and isolated endocrine cells within the non-neoplastic glandular mucosa following neo-adjuvant treatment of oesophageal and oesophago-gastric junction adenocarcinomas. This finding most likely reflects persistence of normal endocrine cells (pseudo-hyperplasia) in the setting of glandular atrophy, analogous to the changes seen in patients with atrophic gastritis. Pathologists should be aware of this therapeutic effect so that endocrine cells are not mistaken for residual neoplastic elements, and neo-adjuvant therapy should be considered in the differential diagnosis of gastro-oesophageal endocrine cell pseudo-hyperplasia.

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Competing interests: None declared.

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