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World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastrointestinal Oncology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
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Baishideng Publishing Group Inc
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TOPIC HIGHLIGHT

Dr. Edward Yu, *Series Editor*

Esophageal cancer management controversies: Radiation oncology point of view

Patricia Tai, Edward Yu

Patricia Tai, Department of Oncology, Division of Radiation Oncology, Allan Blair Cancer Center, University of Saskatchewan, Regina, SK S4T 7T1, Canada
Edward Yu, Department of Oncology, Division of Radiation Oncology, Western University, London, ON N6A 4L6, Canada
Author contributions: All authors designed, wrote and approved the final version of manuscript.
Correspondence to: Dr. Edward Yu, Department of Oncology, Division of Radiation Oncology, Western University, 790 Commissioner Road East, London, ON N6A 4L6, Canada. edward.yu@lhsc.on.ca
Telephone: +1-519-6858650 Fax: +1-519-6858627
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Abstract

Esophageal cancer treatment has evolved from single modality to trimodality therapy. There are some controversies of the role, target volumes and dose of radiotherapy (RT) in the literature over decades. The present review focuses primarily on RT as part of the treatment modalities, and highlight on the RT volume and its dose in the management of esophageal cancer. The randomized adjuvant chemoradiation (CRT) trial, intergroup trial (INT 0116) enrolled 559 patients with resected adenocarcinoma of the stomach or gastroesophageal junction. They were randomly assigned to surgery plus postoperative CRT or surgery alone. Analyses show robust treatment benefit of adjuvant CRT in most subsets for postoperative CRT. The Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study (CROSS) used a lower RT dose of 41.4 Gray in 23 fractions with newer chemotherapeutic agents carboplatin and paclitaxel to achieve an excellent result. Target volume of external beam radiation therapy and its coverage have been in debate for years among radiation oncologists. Pre-operative and post-operative target volumes are designed to optimize for disease control. Esophageal brachytherapy is effective in the palliation of dysphagia, but should not be given concomitantly with chemotherapy or external beam RT. The role of brachytherapy in multimodality management requires further investigation. On-going studies of multidisciplinary treatment in locally advanced cancer include: ZTOG1201 trial (a phase Ⅱ trial of neoadjuvant and adjuvant CRT) and QUINTETT (a phase Ⅲ trial of neoadjuvant *vs* adjuvant therapy with quality of life analysis). These trials hopefully will shed more light on the future management of esophageal cancer.

Key words: Radiotherapy; Chemotherapy; Esophagus; Cancer; Treatment

Core tip: Esophageal cancer treatment has evolved from single modality to trimodality therapy. There are some controversies of the role, target volumes and dose of radiotherapy (RT) in the literature over decades. Esophageal brachytherapy is effective in the palliation of dysphagia, but should not be given concomitantly with chemo or external beam RT. On-going studies include: ZTOG1201 trial (a phase Ⅱ trial of neoadjuvant and adjuvant chemoradiation) and QUINTETT (a phase Ⅲ trial of neoadjuvant *vs* adjuvant therapy). These trials hopefully will shed more light on the future management of esophageal cancer.

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INTRODUCTION

Over the past 20 years there have been many significant

changes in the management of esophageal cancer. This disease has shown remarkable changes in histology of adenocarcinoma on the rise over squamous cell carcinoma, and in epidemiology with concentration of tumors adjacent to the gastro-esophageal junction (GEJ). Esophageal cancer has evolved from single modality treatment in the past to trimodality treatment currently. Radiotherapy (RT) has been part of the integral management of esophageal cancer for decades. Greater understanding of the natural history has influenced the approach to diagnosis and to treatment options. Appreciation of the need for multidisciplinary approach in treatment planning has reflected the important role of various treatment modalities. There are different clinical practices of combined treatments and controversies often arise. This is aggravated by the difficulty to conduct large-scale randomized trials since many patients are elderly with multiple co-morbidities. A Medline search revealed a limited number of randomized studies in the past decade. The present article reviews RT in the multimodality management of esophageal cancer, with emphasis on the controversy of RT target volume, and radiation dose. A few examples of the controversies are listed here in this section.

The challenges to treat elderly patients with esophageal cancers had been reported[1]. During recent years, the curative potential of RT *vs* surgery for esophageal cancer was investigated in randomized trials. A metaanalysis showed that overall survival (OS) was equivalent between surgery and definitive chemoradiotherapy (CRT) (HR = 0.98 95%CI: 0.8-1.2, P = 0.84)[2]. There was a trend to more cancer related deaths in the definitive RT+/-chemotherapy (chemo) arms [HR = 1.19 (0.98-1.44), P = 0.07], predominantly due to a higher risk of loco-regional progression [HR = 1.54 (1.2-1.98), P = 0.0007] but treatment related mortality was lower in the conservative arms [HR = 0.16 (0-0.89), P = 0.001]. The similar outcome in survival suggests that the safer approach of CRT is a reasonable choice especially in comorbid patients with esophageal squamous cell carcinoma.

For patients with less advanced esophageal cancer patients, the benefit of neoadjuvant therapy is still unclear. However, due to the significant under staging of T2 N0 patients (50% in the Johns Hopkins series), the authors recommend neoadjuvant therapy to all cT2N0 patients before operation[3].

ROLE OF EXTERNAL BEAM RT

Surgery has been considered the standard of care for stage I resectable esophageal cancer with 5 year survival of 60%-70%, stage Ⅱ 40%, stage Ⅲ 20%[4]. RT will be discussed in the following sections including its role with chemo before surgery (abbreviated as S here), after surgery with and without chemo, and whether RT is needed in the trimodality management: (1) C + S *vs* S; (2) CRT + S *vs* S; (3) S *vs* S + RT; (4) S *vs* S + CRT; (5) CRT + S *vs* S + CRT; and (6) CRT + S *vs* CRT.

C + S vs S: Perioperative chemo without RT

A landmark study confirmed that this treatment improves survival. The 503-patient United Kingdom National Cancer Research Institute Medical Research Council Adjuvant Gastric Infusional Chemo trial is the first randomized trial to demonstrate a conclusive survival benefit of perioperative chemo for patients with resectable adenocarcinoma of the stomach, GEJ, and lower esophagus, compared with surgery alone[5]. Epirubicin, cisplatin, and infused 5-fluorouracil (ECF) decreased tumor size and stage and hence significantly improved progression-free and overall survival. However, infusional chemo is difficult to administer[6]. In this study, RT is not required. Opinions arise regarding the relative efficacy of CRT *vs* chemo alone in the multimodality management setting. A multicenters randomized Trial of Preoperative therapy for Gastric and Esophagogastric Junction Adenocarcinoma from National Cancer Institute of Canada, European Organization for Research and Treatment of Cancer (EORTC), and Trans-Tasman Radiation Oncology Group is underway to compare preoperative CRT using 45 Gray (Gy) with preoperative chemo alone for GEJ and gastric adenocarcinoma[7]. The chemo regimen in both arms is ECF or EC Xeloda. The result of this trial may offer further insight to the above dilemma that clinicians often have.

CRT + S vs S: Does neoadjuvant CRT improve survival?

The use of neoadjuvant CRT has become an increasingly used treatment approach[8]. Tables 1 and 2 summarizes the potential benefit of preoperative therapy[9]. A few key randomized clinical trials of preoperative CRT with surgery compared to surgery alone are discussed below. Caution to compare across studies is advised. There is great variation of RT dose schemes and the optimum treatment schedule is not clear.

Nygaard *et al*[10] showed that 3-year survival was significantly higher in the pooled groups receiving RT as compared with the pooled groups not receiving RT. Comparison of the groups having pre-operative chemotherapy with those not having chemo showed no significant difference in survival.

Walsh *et al*[11] employed two courses of 5-flurouracil (5-FU), 15 mg/kg daily for five days, and cisplatin, 75 mg/m^2 on day 7. This cycle was repeated in week 6. RT of 40 Gy/15 fractions (f)/3 wk was administered.

Bosset *et al*[12] with the Fondation Française de Cancérologie Digestive and EORTC Gastrointestinal Tract Cancer cooperative Group conducted the largest study of its kind with 282 patients. They gave two courses of cisplatin, at a dose of 80 mg/m^2 on 0 to 2 d before each course of RT. The target of RT was the macroscopic tumor and enlarged lymph nodes, if any, surrounded by 5-cm proximal and distal margins and a 2-cm radial margin. After a median follow-up of 55.2 mo, no significant difference in OS was observed; the median survival was 18.6 mo for both groups. Although median or OS

Table 1 Important randomized trials for preoperative chemoradiation *n* (%)

Ref.	*n*	Histology	Treatment	R0	pCR	Op mortality	MS	3 YS	Locoregional failure
Nygaard *et al*[10], 1992		Sq	S	37%	-	5 (3.4)	Approximately 0.6 yr	Approximately 9%	-
			CB → S	41%		6 (4.0)	Approximately 0.7 yr	Approximately 2%	
			R → S	40%		4 (2.7)	Approximately 0.9 yr	Approximately 20%	
			CB + R → S	55% (Gp 4 *vs* 1, *P* = 0.08)		8 (5.4)	Approximately 0.7 yr	Approximately 18%	
Walsh *et al*[11], 1996	113	A	CF + R → S	-	25%	5 (10.4)	16	32%	-
			S	-	0%	2 (3.7)	11 mo	6%	-
							P = 0.01	*P* = 0.01	
Bosset *et al*[12], 1997	282	Sq	C + R → S	-	26%	17 (12.3)	18.6 mo	36%	-
			S	-	0%	5 (3.6)	18.6 mo	34%	-
								See text	
Urba *et al*[13], 2001	100	75% A	CFV + R → S	90%	28%	1 (2.1)	16.9 mo	30%	19%
		25% Sq	S	90%	0%	2 (4)	17.6 mo NS	16%	42%
									P = 0.02
Burmeister *et al*[14], 2005	256	37% Sq	CF + R → S	80%	16%	5 (4.8)	22.2 mo	35%	15%
		62% A	S	59%	0%	6 (5.5)	19.3 mo	30%	19%
		1% mixed/ other						See text	
Tepper *et al*[15], 2008	56	25% Sq	CF + R → S	-	33%	0 (0)	4.5 yr	39%	13%
		75% A	S		0%	1 (3.8)	1.8 yr	16%	15%
							P = 0.002	5 YS	
Cao *et al*[9], 2009	366	Sq	CFM → S	87%	1.7%	0%	Approximately 42 mo	Approximately 69%	-
			R → S	98%	15%	0%	Approximately 42 mo	69%	
			CFM + R → S	98%	22%	0%	Approximately 60 mo	74%	
			S	73%	0%	0%	Approximately 42 mo	53%	
								P = 0.013	
van Hagen *et al*[16], 2012	366	23% Sq	JT + R → S	92%	29%	6 (4)	49.4 mo	58%	-
	T1-3	75% A	S	69%	0%	8 (4)	24 mo	44%	
	N0-1	2% other						*P* = 0.03	
	M0								

-: Not reported; A: Adenocarcinoma; B: Bleomycin; C: Cisplatin; F: 5-flurouracil; Gp: Group; J: Carboplatin; M: Mitomycin; MS: Median survival; NS: Non-significant; Op: Operative mortality using number of patients actually operated as denominator; pCR: Pathological complete response; R: RT; R0: No residual tumor; S: Surgery; Sq: Squamous cell carcinoma; T: Paclitaxel; V: Vinblastine; YS: Year survival.

Table 2 Pros and cons of pre-operative therapy for esophageal cancer

Pre-op therapy	Pros	Intact vascular supply allowing for potential improved oxygenation for radiotherapy
		Smaller radiotherapy volume
		Potential tumor downstaging
		Sterilization of tumor bed in preparation for surgery
		Improve resectability
	Cons	Treatment decision based on clinical stage, may over-treat patients
		Narrow window for surgical resection post CRT, may increase surgical complications with pre-op CRT
		Dysphagia and issue of nutrition support due to tumor and treatment

CRT: Chemoradiation therapy.

were not significantly different, there was a significant difference in the proportion of deaths that were due to esophageal cancer in the 2 groups (87 of 101 patients who had surgery alone *vs* 69 of 102 patients who received combined treatment CRT and surgery, $P = 0.002$). As compared with the group treated with surgery alone, the group treated preoperatively had longer disease-free survival ($P = 0.003$), a longer interval free of local disease ($P = 0.01$), and a higher frequency of curative resection ($P = 0.017$). However, there were more postoperative deaths ($P = 0.012$) in the group treated preoperatively with CRT.

In the study of Urba *et al*[13], the preoperative CRT arm had cisplatin 20 mg/m^2 per day on days 1-5 and 17-21, 5-FU 300 mg/m^2 per day on days 1-21, and vinblastine 1 mg/m^2 per day on days 1-4 and 17-20. The tumor volume was treated with 5-cm cephalo-caudad margins and 2-cm radial margins by 1.5 Gy twice daily to 45 Gy. One patient had a microscopic positive margin in the surgical specimen and received postoperative RT. This study did not give postoperative RT for patients with positive nodes, but would use it for positive margins of resection.

Burmeister *et al*[14] used 80 mg/m^2 cisplatin intravenously on day 1 followed by 800 mg/m^2 per day 5-FU given intravenously on days 1-4. RT 35 Gy/15 f per 3 wk to the midplane, was started concurrently with

chemo. The results were not statistically significant. Neither progression-free survival nor OS differed between groups [HR = 0.82 95%CI: 0.61-1.10 and 0.89 (0.67-1.19), respectively]. The CRT + S group had more complete resections with clear margins than did the surgery-alone group [103 of 128 (80%) *vs* 76 of 128 (59%), $P = 0.0002$], and had fewer positive lymph nodes [44 of 103 (43%) *vs* 69 of 103 (67%), $P = 0.003$]. Subgroup analysis showed that patients with squamous-cell tumours had better progression-free survival with chemoradiotherapy than did those with non-squamous tumours [HR = 0.47 (0.25-0.86) *vs* 1.02 (0.72-1.44)]. However, the trial was underpowered to determine the real magnitude of benefit in this subgroup.

CALGB 9781 shows the benefit of CRT before surgery despite the closure due to poor accrual[15]. Cisplatin 100 mg/m^2 and 5-FU 1000 mg/m^2 per day for 4 d on weeks 1 and 5 concurrent with RT (50.4 Gy/28 f per 5.6 wk) was followed by esophagectomy with node dissection in the trimodality arm. The median survival was 4.48 years *vs* 1.79 years in favor of trimodality therapy over surgery alone (exact stratified log-rank, $P = 0.002$).

Results from a recent multicenter phase Ⅲ randomized trial, Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study (CROSS study) showed that neoadjuvant CRT improved OS compared to surgery alone in patient with resectable (T2-3N0-1M0) esophageal or GEJ cancers[16]. Median survival was 49 mo in the neoadjuvant CRT arm and this seems to be the best median survival results achieved in the literature so far (Table 1). The CROSS study used a lower RT dose with newer chemo agents. The CRT consisted of weekly administration of carboplatin (doses titrated to achieve an area under the curve of 2 mg/mL per minute) and paclitaxel (50 mg/m^2) for 5 wk and concurrent RT (41.4 Gy/23 f per 4.6 wk), followed by surgery. The RT volume is also modest: the planning target volume (PTV) employed a proximal and distal margin of 4 cm around the gross tumor volume (GTV), and in case of tumor extension into the stomach, a distal margin of 3 cm was used. A 1.5 cm radial margin around the GTV was provided to include the area of subclinical involvement around the GTV and to allow for tumor motion and set-up variations.

Some patients may refuse to have surgery after a clinical complete response (clinCR) to preoperative CRT. From the prospective database of MD Anderson Cancer Center, 61 of the 622 trimodality-eligible patients declined surgery after a clinCR, defined as both endoscopic biopsy showing no cancer and physiologic uptake by positron emission tomography (PET)[17]. Forty-two out of the 61 patients were alive at a median follow-up of 50.9 mo (95%CI: 39.5-62.3). The 5-year overall and relapse-free survival rates were 58.1% ± 8.4% and 35.3% ± 7.6%, respectively. Of 13 patients with local recurrence during surveillance, 12 had successful salvage resection. The authors concluded that although the outcome of 61 patients with clinCR who declined surgery appears reasonable, in the absence of a validated prediction/prognosis model, surgery must be encouraged for all trimodality-eligible patients.

In 2011, Kranzfelder *et al*[18] published a meta-analysis which sought to clarify the benefits of neoadjuvant treatment: there were nine randomized controlled trials involving neoadjuvant CRT *vs* surgery, eight involving neoadjuvant chemo *vs* surgery. The HR for OS was 0.81 (95%CI: 0.70-0.95, $P = 0.008$) after neoadjuvant CRT and 0.93 (0.81-1.08, $P = 0.368$) after neoadjuvant chemo. Morbidity (HR = 1.03, $P = 0.638$) and mortality (HR = 1.04, $P = 0.810$) rates after neoadjuvant chemo and surgery did not differ from those after surgery alone. However, the 30-d mortality was non-significantly higher with combined treatment.

S vs S + RT: Postoperative adjuvant RT without chemo

Post-esophagectomy adjuvant RT can reduce local recurrence rate[19,20]. Several randomized trials were performed comparing surgery plus postoperative RT (PORT) with surgery alone to clarify the impact of PORT[21,22]. The majority of the evidence has revealed that PORT may improve local disease recurrence but does not confer any survival benefit over surgery alone[23,24]. These trials had limitations: (1) patients were not stratified by stage hence unlikely to detect an improvement in survival in those with high risk features (positive lymph nodes, deeply invading tumors); (2) they often include patients with positive celiac nodes; (3) they include mostly squamous cell carcinomas; and (4) no chemo were given. Adjuvant RT can theoretically treat microscopic disease left behind after surgery to increase local control, but cannot eradicate systemic spread of tumor cells.

Schreiber *et al*[25] performed a retrospective review using the American Surveillance Epidemiology and End Results (SEER) database to analyze whether there was survival benefit to adjuvant RT in stage T3-4N0M0 or T1-4N1M0 esophageal cancer who were definitively treated with esophagectomy. A total of 1046 patients met the selection criteria; 683 (65%) received surgery alone and 363 (34.7%) received PORT. For stage Ⅲ esophageal carcinoma (T3N1M0 or T4N0-1M0), 346 patients underwent surgery alone and 231 patients received PORT. Use of PORT resulted in an improvement in median OS from 15 to 19 mo and an improvement in 3-year OS from 18.2% to 28.9% ($P < 0.001$), respectively. This benefit was present for both squamous cell and adenocarcinoma. One limitation of the SEER data is the lack of information on use of chemo, so the benefit could be effect of CRT.

S vs S + CRT: Postoperative adjuvant CRT

Some studies[26,27] addressed the impact of PORT with chemo on node-positive esophageal carcinoma, and found a survival benefit. The randomized adjuvant CRT trial, Intergroup trial (INT 0116) enrolled 559 patients with resected adenocarcinoma of the stomach or GEJ. They were randomly assigned to surgery plus postoperative CRT or surgery alone[28]. The adjuvant arm used 425

Table 3 Pros and cons of post-operative therapy for esophageal cancer

Post-op therapy	Pros	Treatment decision based on true pathologic stage, avoid CRT in patient who may not require it
		Accurate assessment of disease extent to allow delineation of disease involvement
		Immediate relief of dysphagia due to tumor
	Cons	Difficulty to delineate RT target volume
		Large RT therapy volume and difficulty in RT planning
		Potential decrease in oxygenation to tumor bed due to postoperative tissue alteration in vascular supply
		Inability to assess RT or chemo tumor response
		May preclude the use of postoperative CRT for those patients with reduced functional status postoperatively

CRT: Chemoradiation therapy; RT: Radiotherapy.

mg/m^2 of 5-FU, plus 20 mg/m^2 of leucovorin per day, for 5 d, followed by 45 Gy/25 f per 5 wk of daily RT, with modified doses of 5-FU and leucovorin on the first 4 and the last 3 d of RT. A month after the completion of RT, two 5-d cycles of 5-FU (425 mg/m^2 per day) plus leucovorin (20 mg/m^2 per day) were given 1 mo apart. Hence a total of 4 mo cycles of adjuvant chemo was given. Twenty percent of the patients had GEJ adenocarcinoma. Subset analyses show robust adjuvant treatment benefit in most subsets.

CRT + S vs S + CRT: Preoperative vs postoperative therapy

Tables 2 and 3 compare the advantages of preoperative *vs* postoperative therapy[29,30]. There are no well performed randomized trials to compare the outcome of pre- against post-operative therapy with modern treatment staging and treatment techniques. Neoadjuvant treatments can be started immediately targeting any micro-metastatic deposits without allowing time for further cancer growth. The exact disease staging often cannot be firmly assessed at the preoperative circumstances.

Further research of the multidisciplinary management for patients with locally advanced esophageal cancer is warranted. The approach is currently being explored in two countries: China and Canada. In China the study has been carried out by investigators of the ZTOG1201 trial, a multicenter phase Ⅱ trial of neoadjuvant and adjuvant CRT in locally advanced esophageal cancer (NCT01463501)[31]. In Canada, this is undertaken by investigators of the QUINTETT phase Ⅲ trial (NCT00907543) of neoadjuvant *vs* adjuvant therapy in locally advanced esophageal cancer trial including quality of life[32]. Results of these trials can potentially provide further insight on the impact of trimodality therapy on the management of locally advanced esophageal cancers.

CRT + S vs CRT: Does surgery add to CRT?

The omission of surgery would leave residual disease behind and therefore surgery theoretically should contribute to treatment success. There were clinical trials comparing neoadjuvant CRT followed by esophagectomy to definitive CRT. Stahl *et al*[33] randomized 86 patients with advanced squamous cell carcinoma of the esophagus for neoadjuvant CRT of cisplatin, leucovorin, etoposide and 40 Gy RT followed by esophagectomy, compared to 86 patients treated with same chemo but 65 Gy RT and no surgery. The median survival was 16 and 15 mo with and without surgery, respectively. The 2-year survival rate was 40 and 35 mo with and without surgery, respectively. HR was 0.83 (0.54, 1.23) and was non-significant.

The other trial was performed by Bedenne *et al*[34]. Their trial randomized 129 patients with advanced squamous cell carcinoma of esophagus for neoadjuvant CRT of cisplatin, 5-FU, 46 Gy RT followed by esophagectomy, comparing with 130 patients treated with the same chemo but 66 Gy without surgery. The median survival was 18 and 19 mo with and without surgery, respectively. The 2-years survival was 34 and 40 mo with and without surgery, respectively. The HR was 0.88 (0.59, 1.31) and was non-significant.

In a Phase Ⅱ trial in Radiation Therapy Oncology Group (RTOG 0246)[35], definitive CRT employed induction 5-FU (650 mg/m^2 per day), cisplatin (15 mg/m^2 per day), and paclitaxel (200 mg/m^2 per day) for two cycles, followed by concurrent CRT with 50.4 Gy/28 f and daily 5-FU (300 mg/m^2 per day) with cisplatin (15 mg/m^2 per day) over the first 5 d. Salvage surgical resection was considered for patients with residual or recurrent esophageal cancer who did not have systemic disease. The study was designed to detect an improvement in 1-year survival from 60% to 77.5% (α = 0.05; power = 80%). Only 71% 1-year survival was achieved among the 43 patients enrolled from September 2003 to March 2006.

These trials had low to moderate sample size, short follow up, and the RT dose in the nonsurgical arm was above 60 Gy. This was concluded, in the meta-analysis of Kranzfelder *et al*[18] that no trials demonstrated a significant survival benefit of definitive CRT compared with neoadjuvant treatment followed by surgery, however the likelihood of R0 (no residual tumor) resection was significantly higher after neoadjuvant CRT (HR = 1.15, P = 0.043).

In the specific scenario of T4 esophageal cancers, defined as a tumor that invades neighboring structures (*e.g.*, aorta, trachea, bronchus, and lung), are usually considered inoperable despite recent advances in surgical techniques. CRT + S is superior to CRT with respect to local control and short-term survival although CRT-S is associated with relatively higher perioperative mortality and morbidity[36]. On the other hand, it is sometimes difficult to achieve local control with CRT and the treatment often

results in fistula formation, though a complete response to CRT is often associated with better prognosis. Admittedly, the difference in the survival rate between the two modalities is marginal at long-term follow-up due to operative morbidity and inadequate control of distant metastasis in CRT-S. Randomized controlled trials involving large population samples are needed to define the standard treatment for T4 esophageal cancer.

ROLE OF BRACHYTHERAPY

Esophageal brachytherapy alone is no longer used for curative situation because it can only effectively treat cancer within 1 cm radius, and unable to reach the adjacent lymphatic drainage at risk. If external beam RT is not possible, high dose rate (HDR) brachytherapy 6 Gy for 3 f or 8 Gy for 2 f at 1 cm from the center of the source axis can palliate dysphagia[37]. It should not be given concomitantly with chemo or external beam RT. The toxicity was reported by RTOG 92-07 study[38]. This phase I / II study planned to give 50 Gy/25 f per 5 wk of external beam RT followed 2 wk later by brachytherapy (either HDR 5 Gy during weeks 8, 9, and 10, for a total of 15 Gy, or low-dose-rate 20 Gy during week 8). Chemo was given during weeks 1, 5, 8, and 11, with cisplatin 75 mg/m^2 and 5-FU 1000 mg/m^2 per 24 h in a 96-h infusion. The final analysis showed severe toxicity, including treatment-related fistulas, occurred in 6/49 (12% patients, 14% among those starting brachytherapy) within 7 mo of brachytherapy.

HDR brachytherapy before external beam RT and chemo as a boost in the treatment of patients with esophageal cancer was reported to be safe in a single institution study[39]. Further investigation on the role of HDR brachytherapy boost treatment in multimodality management is needed. Other ways of brachytherapy for esophageal cancer palliation was studied, in the form of self expandable stent loaded with radioactive seeds of low dose rate brachytherapy. In a single institution small pilot study, 53 patients were randomized to an I -125 loaded stent or a conventional stent[40]. Systemic therapy was allowed for both the treatment and control group. The benefit for relief of dysphagia was significant after 2 mo ($P < 0.05$). The stent restenosis occurred later in the RT stent group than in the control group (4.75 mo *vs* 2.00 mo) ($P < 0.05$). In RT stent group, median OS was 7 mo (95%CI: 5.0-10.0) and mean OS was 8.3 mo (95%CI: 6.36-10.21). In control group, median OS was 4 mo (95%CI: 2.0-4.0) and mean OS was 3.5 mo (95%CI: 2.720-4.16) ($P < 0.001$, log-rank test).

TARGET VOLUME OF EXTERNAL BEAM RT

The ERT treatment volume for esophageal cancer is controversial. For example, distal esophageal adenocarcinomas at the GEJ may be treated with esophageal cancer RT portal instead of stomach cancer RT portal. The following section will discuss the preoperative and postoperative RT target volumes.

Preoperative and definitive RT

Tai *et al*[41] noted a great variability in target volume delineation. In the absence of a general consensus guideline, this could be due to practice variations among oncologists in individual cases. Esophageal cancer can extend submucosally in the longitudinal direction for a considerable distance. Miller *et al*[42] reported that in 15% of cases, microscopic longitudinal spread at greater than 6 cm from the primary lesion can occur. However, this cannot become the clinical tumor volume (CTV) since with expansion, the PTV would be very long cranio-caudally.

Recently lean management has been used in health care. A study from Loyola University Medical Center indicates the feasibility of applying the "plan-do-check-act" (PDCA) cycle to assess competence in the delineation of individual organs, and to identify areas for improvement[43]. With testing, guidance, and re-evaluation, contouring consistency can be obtained. The PDCA approach will ensure more accurate treatments and continual quality improvement.

In RTOG 9405, the initial target volume (50.4 Gy) encompassed 5 cm margin for the superior and inferior borders[44]. The lateral, anterior, and posterior borders of the field were 2 cm or more beyond the borders of the primary tumor. The tumor size was defined by endoscopic ultrasound (EUS), barium swallow, or computed tomography (CT) scan (whichever was larger). The primary and regional lymph nodes were included. For tumors of the cervical esophagus, the supraclavicular lymph nodes were included. A separate photon or electron boost to the supraclavicular lymph nodes was allowed to bring the total dose to 50.4 Gy. Patients randomized to the high-dose arm received a cone down of 14.4 Gy to attain a total dose of 64.8 Gy. The intent of the cone down was to treat the primary tumor only, not the regional primary lymph nodes. The superior and inferior borders of the field were decreased to 2 cm beyond the tumor. The lateral, anterior and posterior borders were the same as the initial target volume.

Image-guided RT is used in many North American Centers nowadays. The experience in MD Anderson Cancer Center showed large (> 1 cm) inter-fractional displacements in the GEJ in the superior-inferior (especially inferior) direction was not accounted for when skeletal alignment alone was used for patient positioning[45]. Because systematic displacement in the superior-inferior direction had dosimetric impact and correlated with tidal volume, better accounting for depth of breathing is needed to reduce inter-fractional variability. Patients are also advised to be nil by mouth 3 h before planning CT or daily RT so that the stomach is empty.

To summarize (Figure 1A): (1) GTV includes visible tumor on CT, barium swallow, EUS, and PET scans; (2) CTV: GTV + 1 cm radially and 3-4 cm longitudinally. One may edit for anatomic barriers: vertebral bodies, ves-

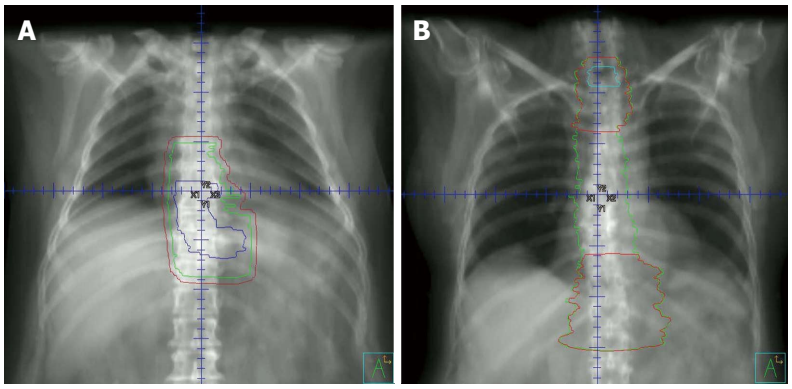

Figure 1 Radiation field for a lower esophageal cancer. A: Pre-operative with minimal involvement of gastro-intestinal junction: celiac nodes are not covered. Intensity modulated radiotherapy is used. Blue: Gross tumor volume; Green: Clinical target volume; Red: Planning target volume; B: Post- operative with involvement of gastro-esophageal junction. Intensity-modulated radiotherapy treatment. Blue: Anastomosis; Green: Clinical target volume; Orange: Clinical target volume concomitant boost, planning target volume not shown.

sels and heart. Supraclavicular nodes are covered for cervical esophagus only. Coeliac nodes are covered for lower esophageal lesions; (3) PTV: CTV + 1 cm; and (4) Field borders: generally 2 cm radial, 4-5 cm longitudinal margins. For cervical esophageal tumors, the superior field border is just below larynx. If celiac nodes to be covered, the field goes down to the bottom of T12 or L1.

Postoperative target volume

In postoperative adjuvant RT, a retrospective study of 72 high-risk patients (T3, T4, nodes positive, with or without margin involvement) treated at the London Regional Cancer Centre from 1989 to 1999 addressed the controversy whether the anastomotic site needs to be included)[46,47]. Positive/close margins were found in 34 (49%) patients. Median follow-up was 30.5 mo (range 3.4-116.3 mo). Anastomosis recurrence rates were 29% with small volume and 0% with extended volume RT (P = 0.041). Local and regional relapse occurred in 74.2% of patients treated with small volume RT compared to 15.4% in patients treated with extended volume RT (P < 0.001). After adjusting for resection margin status, the local control benefit of extended volume RT remained significant (P = 0.003).

To define the target volume, use of PET or PET/CT, alone or in combination with other methods, may be better to evaluate how far a tumour has spread (staging), whether it has responded to treatment (restaging), or detection of recurrences[48]. However, a German review of 48 studies found no strong evidence that PET, alone or in combination with CT, increases survival, improves quality of life, or results in fewer operations or diagnostic interventions[49].

To summarize (Figure 1B): (1) CTV: The tumor bed and the lymphatic drainage at risk (peri-esophageal lymph nodes and regional lymph nodes). For GEJ, the celiac nodes (around T12-L1) may need to be included; (2) PTV: CTV + 1 cm radial and longitudinal margin. The superior margin of the PTV will include the surgical anastomotic site (labeled with radio-opaque clips) proximally with 2 cm margin. The inferior margin of the field will be 5 cm beyond the previous GTV location. Lateral, anterior, and posterior borders will be 2 cm beyond the lateral borders of the tumor bed and regional lymph nodes, except if tumor bed is close to vertebral body, CTV will be on the bony surface. For the GEJ primaries, the celiac nodes (around T12-L1) may need to be included. 36-38 Gy in 28 fractions is delivered including the anastomosis. The tumor bed only should be boosted (simultaneous boost) to 50.4 Gy/28 f per 5.5 wk, together with the anastomosis if the margin is close or positive; and (3) Field borders-superiorly at about T1 to cover the anastomosis, inferiorly to L2-3 if celiac node needs to be covered.

EXTERNAL BEAM RT DOSE FRACTIONATION

Herskovic *et al*[50] (RTOG 85-01) randomized 121 patients to either 50 Gy with concurrent (75 mg/m^2) and 5-FU (1 g/m^2 per 24 h × 4 d) starting with RT for 4 cycles *vs* 64 Gy alone (Table 4). At 5 years, 27% of the combined modality patients were alive *vs* none of those in the RT alone group. For the combined modality, 27% patients had persistent disease and an additional 16% developed local recurrence, compared to 40% and 24% respectively in the RT alone group (P < 0.01). The patients who received combined treatment also had fewer distant recurrences (22% *vs* 38%, P < 0.005). A higher RT dose, 64 Gy, cannot make up for the combined benefit of CRT. However, severe and life-threatening side effects occurred in 44 percent and 20%, respectively, of the patients who received combined therapy, as compared with 25 percent and 3 percent of those treated with RT alone.

Researchers then started to investigate if high RT dose combined with chemo can further increase survival. In the Intergroup 0123 (RTOG 94-05) trial[44] the 218 eligible patients were randomized to 64.8 Gy *vs* 50.4 Gy combined with 4 mo cycles of cisplatin and 5-FU. There was no significant difference in median survival (13.0 mo *vs* 18.1 mo), 2-year survival (31% *vs* 40%), or locoregional

Table 4 Randomized trials for definitive chemoradiation therapy

Ref.	*n*	Histology	Treatment	MS	2 yr OS	Locoregional failure
Herskovic *et al*[50], 1992	121	88% Sq	CF + R 50 Gy	12.5 m	38%	43%
		12% A	R 64 Gy	8.9 m	10%	64%
					$P < 0.001$	local recurrence + persistent primary
Minsky *et al*[44], 2002	218	86% Sq	CF + R 50.4 Gy	18 m	40%	52%
		14% A	CF + R 64.8 Gy	13 m	31% (NS)	56%

A: Adenocarcinoma; C: Cisplatin; F: 5-flurouracil; MS: Median survival; NS: Non-significant; R: RT; Sq: Squamous cell carcinoma.

Table 5 Complications of radiotherapy to esophagus and their management

Acute complications
Skin erythema: 0.5% hydrocortisone, flamazine cream
Hair loss: no treatment
Mucositis, odynophagia, loss of appetite, fatigue, generalized weakness, dysphagia, dehydration, malnutrition, intestinal obstruction: intravenous hydration, xylocaine viscus, feeding tube
Pneumonitis: prednisone, oxygen
Spinal cord L'hermitte sign: no treatment
Larynx hoarseness: prednisone
Fistula/erosion of great vessels, esophageal perforation: consult thoracic surgeons
Chronic complications
Fibrosis/hyperpigmentation of skin: no treatment
Lung fibrosis: oxygen
Esophageal stricture: begins at 3-4 mo. Incidence: 50 Gy 0.8%, 60 Gy 0.6%; 60 Gy + chemo 12%. Treat by dilatation and/or stent
Peptic ulcer: proton pump inhibitor
Chronic enteritis: anti-diarrhoeal, aminosalicylates, pentoxifylline and tocopherol, cholestyramine, antibiotics, corticosteroids, hyperbaric oxygen
Spinal cord myelopathy: hyperbaric oxygen, anticoagulation

failure and locoregional persistence of disease (56% *vs* 52%) between the high-dose and standard-dose arms. Although 11 treatment-related deaths occurred in the high-dose arm compared with 2 in the standard-dose arm, 7 of the 11 deaths occurred in patients who had received 50.4 Gy or less. When comparing the high-dose arm with the low-dose arm, there was a significant prolongation of treatment time due to toxicity interruptions, and less 5-FU delivered doses.

To summarize the studies for esophageal cancer, when concurrent CRT is used without surgery, 54 Gy is recommended, although there are no firm data to support this[51]. In postoperative setting, a large elective volume (PTV1) should include the anastomosis even if the resection margins are adequate, 36-38 Gy in 28 fractions. The tumor bed should be boosted (simultaneous in field with the above mentioned PTV) to 50.4 Gy/28 f, as well as the anastomosis if the margin is close or positive[46,47]. The simultaneous integrated boost used by Yaremko *et al*[52] showed excellent result. Boost of tumor bed increases RT dose locally while a lower dose can be given to a longer clinical target volume.

COMPLICATIONS

Table 5 summarizes the acute and chronic complications for esophageal RT. To reduce complications, RT treatment modalities used in clinical research studies include 3-dimensional conformal RT (3D-CRT), intensity-modulated RT (IMRT) and proton beam therapy (PBT)[53]. When comparing the three RT modalities in 444 esophageal cancers at different locations, there was a significant increase in postoperative pulmonary complications for 3D-CRT compared to IMRT and for 3D-CRT *vs* PBT but not for IMRT compared to PBT after adjusting for pre-RT diffusion capacity of the lung for carbon monoxide (DLCO). When mean heart dose and mean lung dose (MLD) were added to multivariate analysis after adjusting for pre-RT DLCO and RT modality, the effect of RT modality was no longer significant, whereas MLD became the only significant factor for perioperative pulmonary complications.

Another study showed that IMRT compared to 3D-CRT resulted in significantly higher OS, loco-regional control, and non-cancer related mortality rates among 676 esophageal cancer patients[54].

PBT in treatment of esophageal cancer had few severe toxicities, with encouraging pathologic response and clinical outcomes[55]. It is difficult to justify PBT in esophageal cancers at the present time when there are other competing technologies available such as IMRT and until PBT facilities are more readily available as there are few centers currently in the world.

Another way to reduce complications is volumetric arc modulation. A study reported the comparison of RapidArc (RA) against 3DCRT and IMRT techniques for esophageal cancer[56]. CT scans of 10 patients were included in the study. Single-arc and double-arc RA plans were prepared to deliver 54 Gy to the PTV in 30 f. Target conformity improved with double-arc RA plans compared with IMRT. But RA plans resulted in a reduced low-level dose bath (15-20 Gy) in the range of 14%-16% compared with IMRT plans. The average monitor units needed to deliver the prescribed dose by RA technique was reduced by 20%-25% compared with IMRT technique. Therefore, volumetric arc modulation is also favored for shorter treatment time on the machine couch.

Similarly, tomotherapy significantly reduced dose to normal tissues[57]. Mean lung dose was respectively 7.4 and 11.8 Gy ($P = 0.004$) for tomotherapy and 3D plans. Corresponding values were 12.4 and 18.3 Gy ($P = 0.006$) for cardiac ventricles. Maximum spinal cord dose was respectively 31.3 and 37.4 Gy ($P < 0.007$) for tomotherapy and 3D plans.

FUTURE RESEARCH

Chemo

An important limitation of RT is its difficulty to encompass longitudinal local extension, lymphatic and nodal drainage due to normal tissue tolerance. Future research should focus on better chemo or targeted therapy to complement RT treatment. Unfortunately, epidermal growth factor receptors-targeted agents fail to improve outcomes: Panitumumab in REAL-3 trial[58] or cetuximab in SCOPE1 trial[59]. Concomitant cetuximab, cisplatin, irinotecan, and RT were poorly tolerated in the first North American cooperative group trial (S0414) testing this regimen for locally advanced esophageal cancer as treatment-related mortality approached 10%[60].

An on-going study RTOG 1010 examines the role of trastuzumab (Herceptin)[59]. Arm 1 uses RT (50.4 Gy), paclitaxel, carboplatin, and trastuzumab, followed by surgery 5-8 wk after completion of RT, then maintenance trastuzumab, every 3 wk for 13 treatments. Arm 2 does not have any trastuzumab nor any maintenance drug.

Single agent docetaxel was well tolerated in a phase Ⅱ study in China[61]. There is an on-going multicenter study on combination docetaxel, cisplatin and 5-FU in Japan[62].

A trimodal approach, consisting of a single cycle of induction chemo, CRT containing capecitabine and cisplatin, and surgery, was feasible and effective in patients with resectable esophageal squamous cell carcinoma[63]. In another study, neoadjuvant concurrent CRT with capecitabine and oxaliplatin was found to be well tolerated and effective in patients with locally advanced esophageal cancers[64].

Surgery

Improvements in perioperative management may enhance the outcome. The CRT treatment of esophageal cancer follows the example of mitomycin C and 5-FU combination in anal cancer. Recent rectal cancer research on increasing the time interval to 10-11 wk from end of neoadjuvant CRT to surgery results in the highest rate of pathological complete response for rectal cancer[65]. Similarly, future investigations of esophageal RT may pursue gradually increasing the time interval from the end of neo-adjuvant CRT to surgery to find the optimal time. Currently esophagectomy is performed 2-6 wk after completion of CRT. This will allow patients to recover from side effects of concurrent CRT by having good nutritional support prior to surgery, and to minimize any severe postoperative complications after surgery[66]. A prospective database of 266 patients in the MD Anderson Cancer Center between 2002 and 2008 showed that timing of esophagectomy after neoadjuvant CRT (within 8 wk *vs* > 8 wk) is not associated with perioperative complication, pathologic response, or OS. The authors concluded that it may be reasonable to delay esophagectomy beyond 8 wk for patients who have not yet recovered from CRT[67].

PET scan

Another area of on-going research is the use of PET scan to modify therapy. In the CALGB 80803, PET scan non-responders will cross over to the other chemo regimen[68].

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REVIEW

Endoscopic assessment and management of early esophageal adenocarcinoma

Ghassan M Hammoud, Hazem Hammad, Jamal A Ibdah

Ghassan M Hammoud, Hazem Hammad, Jamal A Ibdah, Division of Gastroenterology and Hepatology, University of Missouri, Columbia, MO 65212, United States
Author contributions: Hammoud GM and Hammad H participated in writing the manuscript; Ibdah JA edited the manuscript.
Correspondence to: Jamal A Ibdah, MD, PhD, Professor, Director, Division of Gastroenterology and Hepatology, University of Missouri, 3635 Vista Avenue, St. Louis, Columbia, MO 65212, United States. ibdahj@health.missouri.edu
Telephone: +1-573-8820482 Fax: +1-573-8844595
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Abstract

Esophageal carcinoma affects more than 450000 people worldwide and the incidence is rapidly increasing. In the United States and Europe, esophageal adenocarcinoma has superseded esophageal squamous cell carcinoma in its incidence. Esophageal cancer has a high mortality rates secondary to the late presentation of most patients at advanced stages. Endoscopic screening is recommended for patients with multiple risk factors for cancer in Barrett's esophagus. These risk factors include chronic gastroesophageal reflux disease, hiatal hernia, advanced age, male sex, white race, cigarette smoking, and obesity. The annual risk of esophageal cancer is approximately 0.25% for patients without dysplasia and 6% for patients with high-grade dysplasia. Twenty percent of all esophageal adenocarcinoma in the United States is early stage with disease confined to the mucosa or submucosa. The significant morbidity and mortality of esophagectomy make endoscopic treatment an attractive option. The American Gastroenterological Association recommends endoscopic eradication therapy for patients with high-grade dysplasia. Endoscopic modalities for treatment of early esophageal adenocarcinoma include endoscopic resection techniques and endoscopic ablative techniques such as radiofrequency ablation, photodynamic therapy and cryoablation. Endoscopic therapy should be precluded to patients with no evidence of lymphovascular invasion. Local tumor recurrence is low after endoscopic therapy and is predicted by poor differentiation of tumor, positive lymph node and submucosal invasion. Surgical resection should be offered to patients with deep submucosal invasion.

Key words: Esophageal adenocarcinoma; High grade dysplasia, endoscopic ultrasound; Gastroesophageal reflux; Barrett's esophagus; Chromoendoscopy; Narrow band imaging; Endoscopic mucosal resection; Radiofrequency ablation

Core tip: This review provides an up-to-date summary of the recent published studies on the use of endoscopic diagnosis and endoluminal management in patients with early esophageal adenocarcinoma, including endoscopic mucosal resection and local ablative techniques. Moreover, the review highlights the significance of this disease and the rising incidence of adenocarcinoma in the United States and western world.

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INTRODUCTION

The incidence of esophageal cancer has been increasing steadily in the United States and the western world, with a remarkable 7-fold increase in incidence in the last 30 years[1]. In fact, it has been the most rapidly increas-

ing cancer in white male population[2]. Unfortunately, the overall 5-year survival for early esophageal adenocarcinoma (EAC) has not improved and remains lower than 15%[3].

According to the National Cancer Institute (NCI), it is estimated that 17990 new cases of esophageal cancer will be diagnosed in the United States in 2013, of which approximately 60% will be adenocarcinomas[4].

The other type of esophageal cancer, esophageal squamous cell cancer continues to be the predominant type of esophageal cancer worldwide, but its incidence has been decreasing in the western countries[5]. Although genetic factors play a role in the pathogenesis of esophageal adenocarcinoma[6]. The recent dramatic increase in the incidence of esophageal adenocarcinoma is likely related to increased prevalence of gastroesophageal reflux disease (GERD)[7], increased obesity[8,9] and *Helicobacter pylori* eradication[10,11].

Reflux injury to the lower esophagus resulting in Barrett's esophagus (BE) seems to be the main precursor for EAC. This usually begins with inflammation (esophagitis), which could result after a period of time into intestinal metaplasia (BE) with increased risk to progress to dysplasia and eventually EAC[12]. In addition to acid reflux, bile acid reflux may also play an important role in the progression from Barrett esophagus to esophageal adenocarcinoma. Bile acids are synthesized from cholesterol and down-regulate caveolin-1 in esophageal epithelial cells through sterol responsive element-binding protein[13]. Caveolin-1 protects squamous epithelial cells. Moreover, bile acids increase reactive oxygen species production and cell proliferation *via* activation of PI-PLCgamma2, ERK2 MAP kinase, and NADPH oxidase NOX5-S, thereby contributing to the development of esophageal adenocarcinoma[14].

BE is two to three times more common in men than in women, and is more common in Caucasians. It is less common in African American and is extremely uncommon in Asians[15]. The risk of progression to adenocarcinoma in nondysplastic BE appears to be small. A recent population based study from the Denmark that followed 11028 patients with BE for a median of 5 years reported an annual risk of EAC of 0.12%[16].

The risk of progression to cancer increases in the presence of dysplasia and is up to 6% in patients with high grade dysplasia (HGD)[17].

Risk factors for progression of BE into cancer include low grade dysplasia (LGD), abnormal DNA ploidy and certain lectin binding patterns. Other biomarkers for progression include aberrant DNA methylation changes, expression of microRNAs, as well as overexpression or loss of expression of p53[18].

Endoscopic therapy with curative intent can only be undertaken when the risk of lymph node metastasis is negligible. It is estimated that the rate of lymph node spread is 0% in case of HGD and 1%-2% in case of intramucosal cancers (IMCs). The rate increases to 22% in case of submucosal invasion[19,20].

Table 1 Paris classification of superficial lesions

Type	Lesion
0-Ⅰ	Protruding/polypoid
0-Ⅰp	Pedunculated
0-Ⅰs	Sessile
0-Ⅱ	Non-protruding/non-excavated
0-Ⅱa	Slightly elevated
0-Ⅱb	Flat
0-Ⅱc	Slightly depressed
0-Ⅲ	Excavated

Protruding (0-Ⅰ), depressed (0-Ⅱc) and excavated (0-Ⅲ) lesions have been identified as carrying a higher risk of submucosal invasion[118].

ENDOSCOPIC DIAGNOSIS OF BE AND EARLY EAC

The diagnosis of BE is usually suspected on forward viewing upper endoscopy and is confirmed with histologic examination. Careful examination by high-resolution forward-viewing white-light endoscopy is recommended[21,22]. In a study by Gupta *et al*[23] post hoc analysis of an enriched study population and experienced endoscopists at tertiary referral centers. The authors showed that Longer time spent inspecting the BE segment (BIT) is associated with increased detection of HGD/EAC. Endoscopists who had an average BIT > 1 min per centimeter of BE detected more endoscopically suspicious lesions. Multiple random biopsies should be obtained from the four quadrants every 2 cm in non-dysplastic BE segments and every 1 cm if there is suspicion or history of dysplasia (Seattle protocol). Any visible nodule or lesion is usually suspicious for dysplasia or malignancy and should be sampled separately. Accurate description of the location, size and endoscopic appearance of the lesion is necessary for planning future therapy. Endoscopic description of lesions is usually done using the Paris classification of superficial neoplastic lesions (Table 1), which can help predict submucosal invasion in the digestive tract[24].

When confirmed histologically, the current standard of care for BE surveillance involves careful inspection using high resolution white light endoscopy with random biopsies of the BE segment according to the Seattle protocol and targeted biopsies of any suspicious areas. Multiple studies have shown that the random biopsy protocol has low sensitivity for the detection of early neoplastic changes in BE and has low adherence among endoscopists (50%)[25,26]. Furthermore, a cost-utility analysis by Gordon *et al*[27] concluded that the endoscopic surveillance of patients with non-dysplastic BE is unlikely to be cost-effective for the majority of patients and depends heavily on progression rates between dysplasia grades unless new technologies improve the quality adjusted survival benefit from the surveillance[27].

Resorting to a "random" biopsy protocol reflects the difficulty to recognize early neoplastic changes in BE. One of the reasons for this is the fact that flat lesions (such as Paris 0-Ⅱa and 0-Ⅱb lesions, Table 1) are by far

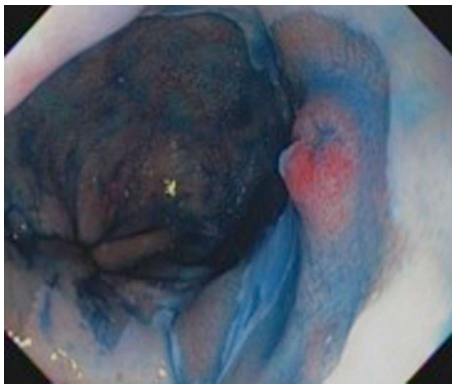

Figure 1 Chromoendoscopy with indigo carmine showing dysplastic nodule in a background of Barrett's mucosa.

the most frequent macroscopic type of neoplastic lesion in BE, and these lesions are typically hard to detect using the standard while light endoscopy[28].

Therefore, there has been major development in image enhancement techniques to improve the detection of early neoplastic lesions in BE. These techniques include detection techniques "red flag" that cover a wide area and help detect a suspicious lesion, and characterization techniques that provide detailed information about a specific area.

DETECTION TECHNIQUES

Dye-based chromoendoscopy

Dye-based Chromoendoscopy consists of spraying the Barrett's mucosa with a dye to better evaluate the microarchitecture of the mucosa to detect early neoplastic changes. Methylene blue was used in the past for this purpose[29-31]; however, it had largely fallen out of favor due to many reasons including difficulty of use and concerns on mutagenesis[32,33]. Indigo carmine is a contrast stain that permeates into the mucosal surface pits and crevices which helps to accentuate any mucosal irregularities[34] (Figure 1). Since it is not absorbed by cells, it does not have safety concerns like methylene blue. A study of 80 patients with suspected BE using high magnification chromoendoscopy with indigo carmine. The yield of intestinal metaplasia (IM) on target biopsies was 97% and 100% for HGD. However, it was not able to distinguish LGD from non-dysplastic intestinal metaplasia[35].

Acetic acid has also been used and provides magnified aspect of the mucosal architecture to help differentiate neoplastic tissue[36]. Curvers *et al*[37] demonstrated that the addition of indigo carmine and acetic acid didn't actually improve the diagnostic yield for early neoplastic lesions in BE compared to high resolution white light endoscopy. Dye-based chromoendoscopy can be labor intensive and is operator dependent and may prolong the procedure. Moreover, it has not been shown to consistently improve the detection of early neoplasia in BE.

Virtual (Dye-less) chromoendoscopy

This includes narrow band imaging (NBI) which uses optical filter to limit the white light illumination to narrow bands of light wavelengths (blue and green), which is predominantly absorbed by hemoglobin and can highlight the capillary network. This results in enhancement of the mucosal vascular and pit patterns and allows visualization of any subtle mucosal irregularities and alteration in vascular patterns concerning for early neoplastic changes[38]. Using pooled data from five studies, Curvers *et al*[39] showed promising results with NBI for detection of early neoplasia in BE with sensitivity of 97%, specificity of 94% and overall diagnostic accuracy of 96%. However, other studies showed a much lower accuracy (71%)[40].

Other virtual chromoendoscopy techniques include Pentax i-Scan and Fujinon intelligent color enhancement. These techniques use post-acquisition image computer reconstruction to enhance mucosal and vascular patterns.

At this time, there is little evidence that chromoendoscopy techniques (both dye-based and dye-less) provide improvements in the characterization and detection of early neoplasia in BE.

Autofluorescence imaging

This technique uses fluorescence radiation following excitation of tissue using light of short wavelengths, which allows differentiation of neoplastic and normal tissue. Autofluorescence imaging (AFI) has been shown to significantly improve the detection of neoplasia in BE; however, the false positive rate is very high (up to 80%)[41]. AFI has also been studied in combination with high resolution endoscopy and NBI, so called Endoscopic Trimodal Imaging (ETMI). In a multicenter randomized trial, ETMI improved the targeted detection of early neoplastic lesions compared to standard video endoscopy. However, the overall histologic yield was not different[42].

Optical coherence tomography and volumetric laser endomicroscopy

Optical coherence tomography produces high quality cross-sectional images of the mucosa based on measuring the rate of backscattering of near-infrared light. This is usually achieved using a probe that is passed through the operative channel of the endoscope. Evans *et al*[43] developed a scoring system for optical coherence tomography (OCT) and reported a sensitivity of 83% and specificity of 75% in the detection of early neoplasia in BE.

Volumetric laser endomicroscopy, the second generation from of OCT, was shown to image the esophageal mucosa at a higher speed and obtain a better quality images[44]. The recent improvements in OCT technology make it a promising technique that can achieve the goal of wide field scanning (detection) as well as characterization of a specific area of concern.

CHARACTERIZATION TECHNIQUES

Endoscopic ultrasound

Endoscopic ultrasound (EUS) may play a little role in the evaluation of patients with HGD or early adenocarci-

noma and is not routinely recommended for evaluation of flat BE segments with HGD[45,46]. A systematic review by Young *et al*[47] showed that the diagnostic accuracy for EUS staging in early EAC was only 65%. A subsequent larger meta-analysis showed better accuracy for EUS in staging T1a and T1b lesions with the area under a receiver operating characteristic curve ≥ 0.93[48]. The use of high-frequency ultrasound catheter probe (miniprobe) can provide a significant better T staging than conventional radial EUS; however, the accuracy is low with both techniques (64% and 49% respectively)[49]. Nevertheless, the National Cancer Comprehensive Network recommends EUS staging prior to proceeding with mucosal resection in the setting of esophageal carcinoma.

Confocal laser endomicroscopy

This is an imaging technique that obtains real-time 1000-fold magnified view of the mucosa, and provides histological information of the target areas (so called virtual histology). Confocal laser endomicroscopy (CLE) could be performed using a dedicated CLE endoscope or miniprobes that can be used with regular large working channel endoscopes (probe-based CLE). A recent study showed that a combination of CLE and while light endoscopy increased the sensitivity for detection of early neoplastic changes compared to white light endoscopy (76% *vs* 34%)[50]. Disadvantages to this technique include that it is expensive, time consuming and requires intensive training.

Spectroscopy

This technique relies on the principle of light interaction with esophageal mucosa to generate a biochemical profile that reflects the cellular architecture. Early results appear to be promising for the real-time detection and diagnosis of esophageal adenocarcinoma with an accuracy of 96%[51]. More recently, Almond *et al*[52] used a novel probe-based endoscopic Raman spectroscopy in *ex vivo* esophageal tissue samples and showed sensitivity of 86% and specificity of 88% for detecting early neoplasia in BE.

The above mentioned enhanced imaging techniques are not widely used in clinical practice due to the limited diagnostic accuracy, high inter-observer variability and high cost. It is also unlikely that these techniques will replace standard high resolution white light endoscopy and random biopsies for surveillance in BE; however, they could play an important role in further characterization and grading of suspicious lesions detected during surveillance exams.

Histopathologic diagnosis

Neoplastic changes in BE can be classified as LGD, HGD, *in situ* (or intraepithelial) carcinoma, IMC and invasive carcinoma[53].

Mucosal lesions are further divided into M1 lesions (or *in situ* carcinoma) when the lesion is limited to the epithelial layer, M2 lesions when the lesion invades the lamina propria and M3 when the lesion invades into but not through the muscularis mucosa layer. Lesions that invade into the submucosal are labeled SM lesions. SM lesion can be further divided into SM1 lesions when the lesion invades into the upper one third of the submucosal, SM2 lesions when the lesion invades the middle third and SM3 lesions when the lesion invades the deep one third of the submucosal layer[54].

Pathologists should carefully evaluate biopsy or resection specimens of esophageal neoplasms to provide details about tumor depth of invasion, tumor differentiation (well, moderate and poorly differentiated), lymphovascular invasion and the presence of tumor invasion at the resection margin. Lymphovascular invasion and poorly differentiated histology increases the risk of lymph node metastasis and these patients should ideally be referred for surgical resection[55].

HGD

HGD is characterized by marked cytological atypia and distorted architecture. Architectural distortion changes include marked crypt crowding, crypt budding and branching. Cytologically, HGD shows cells with marked nuclear pleomorphism, increased N/C ratio, and an increased number of atypical mitoses, particularly in the upper levels of the crypts. Goblet and Paneth cells are usually scarce or absent. Adenomatous (intestinal) dysplasia is the most common subtype but non-adenomatous (foveolar) dysplasia has also been described[56].

Immunohistochemistry staining could help in the diagnosis of HGD. Promising markers include p53 and α-methylacyl coenzyme A racemase but these are not widely used yet[57,58]. Given the significant intraobserver and interobserver variability in the diagnosis of LGD and HGD in BE, most gastrointestinal (GI) societies recommend that a second experienced gastrointestinal pathologist confirm the diagnosis[59]. It is noteworthy that the Japanese and some European pathologists don't use the term HGD and prefer to use the term *in situ* carcinoma for these lesions[60].

Intramucosal carcinoma

IMC invades through the basement membrane to the lamina propria and the muscularis mucosa. It is characterized by atypical cells or complex glands invading into the lamina propria. It is extremely important to differentiate between IMC (or T1a lesion) and carcinoma invading into the submucosa (T1b) as the distinction carries significant implications for the risk of lymph node metastasis and therapy. Such distinction is often difficult to make on biopsy specimens and larger resection specimens such as that resulting from endoscopic mucosal resection (EMR) are more helpful to distinguish between T1a and T1b lesions. In one study, 45% of patients had their final pathological stage changed after EMR compared to pre-EMR forceps biopsies[61]. It also known that most BE usually has double muscularis mucosa layer but this has no impact on the classification or the treatment of Barrett's adenocarcinoma[62].

STAGING OF EARLY ESOPHAGEAL ADENOCARCINOMA

Several modalities have been used to stage esophageal adenocarcinoma. These include EUS, endoscopic mucosal resection with histological assessment and computed tomography/positron emission tomography (CT/PET). EUS and EMR are currently applied as staging tools for early esophageal adenocarcinoma. Early cancer is defined as T1sm1, as beyond this point metastases increases from 1% to 10% for T1sm2 based on a recent consensus[63]. Stage T1a malignancies include lesions confined to the mucosa: M1 (intraepithelial), M2 (lamina propria invasion), or M3 (muscularis mucosa invasion). Submucosal or T1b malignancies are classified into Sm1 (superficial submucosa invasion), Sm2 (invasion to center of submucosa), or Sm3 (invasion to deep submucosa). Mucosal (T1a) malignancies have extremely low risk of local lymph node progression while submucosal invasion (T1b) markedly increases the risk of lymph node metastases[64,65].

EUS

The clinical utility of EUS for staging patients with BE and high-grade dysplasia or intramucosal carcinoma prior to endoscopic therapy has a limited accuracy. The principal role of EUS in evaluating patients with Barrett's-associated dysplasia is to identify patients who may be candidates for endoscopic ablative therapy such as endoscopic mucosal resection and/or photodynamic therapy. EUS has been shown to be superior to computed tomography or magnetic resonance imaging for preoperative staging in patients with high-grade dysplasia and carcinoma. EUS is considered the best tool for T and N staging of esophageal cancer, however, its performance in early Barrett's neoplasia is suboptimal for tumor depth assessment. In a meta-analysis by Puli *et al*[66] the pooled sensitivity of EUS in T1 disease was (88.1%), T2 (82.3%), T3 (89.7%) and T4 (99.2%). EUS can identify nodal spread (N1) or deep tumor invasion (T3) for which it precludes surgical resection. The risk of nodal involvement in early esophageal cancer confined to the mucosa (T1a) ranges between 0% and 3%, and when the lesion extends into the submucosal layer (T1b) this risk approaches up to 30%-50%[67-69]. Tumor size (OR = 1.35 per centimeter, 95%CI: 1.07-1.71) and lymphovascular invasion (OR = 7.50, 95%CI: 3.30-17.07) were the strongest independent predictors of lymph node metastasis[70]. In a retrospective analysis of 135 with HGD (79%) or IMC (21%) who had staging by EUS. Pathologic lymph nodes or metastases were not found by EUS. There were no endosonographic abnormalities noted in any patient with non-nodular mucosa (0/79). However, abnormal EUS findings were present in 14% with nodular neoplasia (five IMC, three HGD)[71]. For patients with nodular neoplasia, endoscopic mucosal resection of the nodule with histological examination had greater utility than staging by EUS. The use of high frequency ultrasound catheter probe (HFP) have been studied in two large studies included 94 and 106 subjects[72,73]. Both studies revealed that HFP is significantly better for lesions localized in the tubular esophagus than the gastroesophageal junction. Moreover, the performance of HFP in assessing submucosal involvement is poor. At this time EUS and HFP staging technique is inadequate for predicting T1-2N0 disease in esophageal adenocarcinoma[74].

Endoscopic mucosal resection

Endoscopic mucosa resection (EMR) has taken a central role in the staging and treatment modality for patients with early esophageal adenocarcinoma, as it allows the pathologist to provide tumor-staging information necessary for an appropriate clinical management decision process. In fact, it is the most accurate staging procedure to assess depth of invasion if full submucosa is provided in the specimen. By providing full thickness of the resected submucosa, pathologists are able to provide a clear histologic depth of the tumor (T staging) and evaluate for lymphovascular invasion. EMR provides better staging for visible lesions than do biopsies alone. Moreover, endoscopic mucosal resection may result in changing the histologic diagnosis in patients with BE with visible and flat neoplasia. In a multicenter study which evaluated 138 patients with BE-related neoplasia who undergone endoscopic eradication therapy showed EMR resulted in a change of the histologic diagnosis in 31.1% patients (upgrades 10.1%; downgrade 21%) with or without visible lesions[75]. At this time, EMR appears to be superior to biopsy for diagnosing and staging superficial esophageal tumors and can substantially modify the diagnostic grade of a lesion. Therefore EMR may facilitate optimal therapeutic decisions by avoiding undertreatment and overtreatment based on inaccurate grading and staging[76].

CT/PET

Early use of PET in the staging of patients with esophageal cancer could facilitate treatment planning and identifying unsuspected distant metastases in up to 20% of patients with a negative metastatic survey by conventional staging[77]. Positron emission tomography detects more distant lymph node and organ metastases compared with conventional diagnostics, allowing a more accurate selection of the most appropriate treatment. CT/PET has inadequate assessment in the superficial esophageal adenocarcinoma. Moreover, the addition of PET to a complete EUS examination did not alter regional-node or celiac-node staging in patients with esophageal cancer[78]. SUVmax ratio was only associated with tumor invasion depth on CT/PET. A recent study evaluated the use of CT/PET in early esophageal adenocarcinoma using a cut-off of 1.48, the sensitivity and specificity of SUVmax ratio for identification of T1a lesions were 43.3% and 80.9%, respectively[79]. Thus more data is needed on the role of CT/PET in early EAC.

ENDOSCOPIC MANAGEMENT OF EARLY ESOPHAGEAL ADENOCARCINOMA

The management of patients with early esophageal cancer

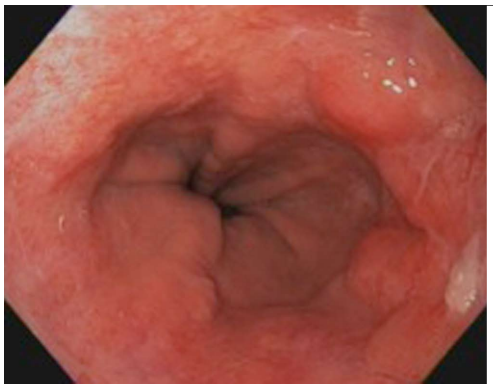

Figure 2 Barrett's esophagus with nodularity concerning for dysplasia or malignancy between 1 and 5 o'clock.

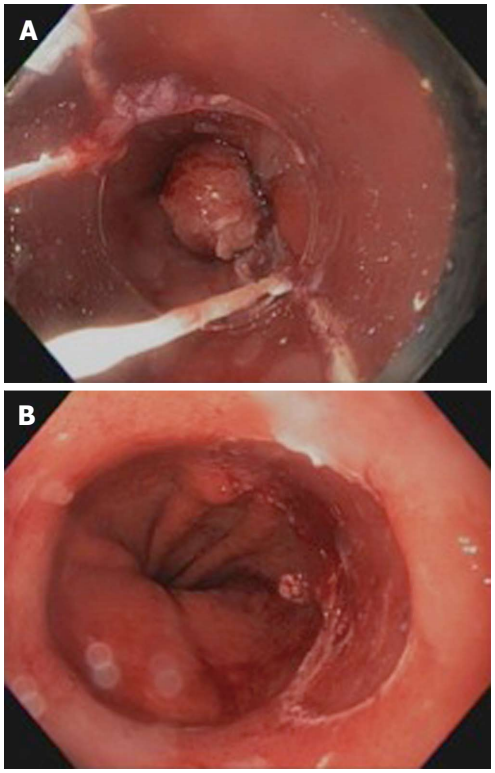

Figure 3 Endoscopic mucosal resection. A: Using Band ligation of Barrette's esophagus nodule; B: Defect after endoscopic mucosal resection using band ligation and resection of Barrett's esophagus nodules.

considered for treatment should take place in a specialty multidisciplinary team including GI pathologist, esophageal surgeon, therapeutic endoscopist, radiologist and oncologist. The endoscopic treatment should commence in high volume tertiary referral centers with availability and expertise in the multiple modalities of endoscopic therapy of BE. Moreover, the center must possess expertise in the management of complications of each modality. The British Society of Gastroenterology recommended a minimum of 30 supervised cases of endoscopic resection and 30 cases of endoscopic ablation should be performed to acquire competence in technical skills, management pathways and complications. Patients with EAC should be informed about the benefits, risks and alternatives of endoscopic and surgical approach. Initially, endoscopic mapping of the Barrett's segment with intestinal metaplasia should be undertaken prior to any endoscopic therapy. The American Gastroenterological Association (AGA) recommends endoscopic eradication therapy for patients with high-grade dysplasia. Risk stratification based on histopathologic assessment should be performed and any nodularity seen on white-light forward viewing upper endoscopy should undergo resection prior to any local ablative therapy (Figure 1). Lymph node metastasis should be excluded. Endoscopic therapy appears to be a good alternative to esophagectomy for patients with low risk pT1b sm1 EAC, on the basis of macroscopic and histologic analyses[55,80]. Data obtained from the Surveillance Epidemiology and End Results database of the NCI to compare cancer-free survival in patients with early esophageal cancer who were either treated with endoscopic therapy (n = 99) or surgical resection (n = 643) did not reveal a difference in esophageal cancer-specific mortality between the two groups[81]. In a population-based analysis, the use of endoscopic therapy for superficial EAC tended to increase from 1998-2009 and the long-term survival of patients with EAC did not appear to differ between those who received endoscopic therapy and those treated with surgery[82]. Several curative modalities are available for local treatment of BE with HGD. Among these modalities are radiofrequency ablation, argon plasma coagulation, thermal laser therapy, cryotherapy and photodynamic treatment. Here we review the efficacy and risks of each modality. Long term outcome of patients with BE and HGD who underwent endoluminal therapy revealed recurrence of intestinal metaplasia occurs in one-third of cases and supports continued endoscopic surveillance even after complete eradication[83].

EMR

Endoscopic mucosal resection provided a primary role in the endoscopic therapy of patients with early EAC (HGD, T1a). EMR should not be attempted if lymph node invasion is suspected. EMR should be performed by an expert therapeutic endoscopist. The principle of EMR is to capture the entire mucosa and submucosa using a suction cup fitted on the tip of the endoscope (Cap-assisted suck and cut or band and cut technique) or lifting the submucosa from the muscularis propria through submucosal injection of saline or indigo carmine (freehand technique). The entire specimen is then excised *en bloc* using a diathermy snare resection or performing multiband mucosectomy[84] (Figures 2 and 3). Total *en bloc* resection is preferred to reduce risk of recurrence and provide accurate histologic assessment. The distinct advantage of EMR over ablative therapy is providing large specimen of resected tissue for histopathologic assessment. One must understand the limitations of EMR include the assessment of base and lateral margin of the tumor resected specimen. The depth of infiltration is better assessed using quantitative micrometric measure in microns

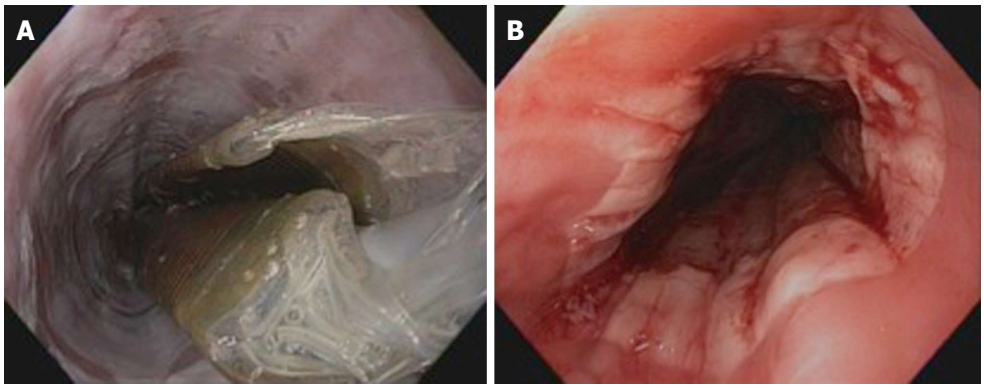

Figure 4 Barrett's esophagus. A: Ablation of Barrett's esophagus using the circumferential balloon catheter; B: Barrett's esophagus after the first round of ablation using the circumferential balloon ablation catheter.

of the depth of submucosal invasion from the bottom of muscularis mucosae. This is deemed to be more accurate than classifying tumor invasion based on depth of submucosal involvement (sm1, sm2, and sm3) as the entire submucosa may not be available in the specimen of all cases[85]. EMR can also be performed in patients with early esophageal adenocarcinoma with previous antireflux surgery[86]. Risk of recurrence after EMR appears low. In one study evaluating 22 patients (16 with HGD), 82% had no evidence of HGD or cancer after a median follow-up of two years[87]. Another long-term follow up study carried in 7 patients for more than 10 years, in 43 for 5-10 years, in 31 for 3-5 years and in 66 for less than 3 years after endoscopic resection. Of the 11 patients who died during the follow up, 10 died of other diseases, only 1 of recurrence of tumor. The 5-year survival rate was 96.2% for early-stage esophageal cancer[88]. Risks of EMR include bleeding, perforation and stricture formation which can occur in up to 37% of cases[61].

Endoscopic submucosal dissection

Endoscopic submucosal dissection is an advanced endoscopic procedure to resect early gastrointestinal neoplasms. It is technically more difficult, carries a high risk when used to treat early esophageal tumors and currently is not widely available in the United States. Studies have been published and reported its efficacy and safety in patients with early EAC[86,89]. In a phase Ⅱ study of endoscopic submucosal dissection for superficial esophageal neoplasms to assess the efficacy and safety of endoscopic submucosal dissection (ESD) in 56 lesions, the *en bloc* resection rate and R0 resection rate were 100% and 94.6%, respectively. The median treatment time for completing the procedure was 69 min (24-168 min)[90]. The rates of adverse events during and after ESD were 22.2% and 53.8%, respectively, but most events were mild. Another study evaluated ESD in combination with radiofrequency ablation in 30 patients with biopsy-proven mucosal adenocarcinoma. Endoscopic follow-up (median 17 mo) showed complete remission of neoplasia in 27/28 (96.4%) patients who underwent successful ESD using waterjet-assisted system[90]. A Meta-analysis by Cao *et al*[91] of endoscopic submucosal dissection *vs* endoscopic mucosal resection for tumors of the gastrointestinal tract showed higher *en bloc* and curative resection rates (OR = 13.87, 95%CI: 10.12-18.99; OR = 3.53, 95%CI: 2.57-4.84) irrespective of lesion size. Subgroup analysis showed higher *en bloc* and curative resection rates with ESD for esophageal, gastric, and colorectal neoplasms, and for lesions of size < 10 mm, 10 mm < 20 mm, and > 20 mm and lower local recurrence. However, ESD was more time-consuming than EMR and showed high procedure-related bleeding and perforation rates (OR = 2.20, 95%CI: 1.58-3.07; OR = 4.09, 95%CI: 2.47-6.80). Similarly, in a previous study evaluating the role of ESD in comparison to EMR in 171 lesions ≤ 20 mm of esophageal cancer (168 were squamous-cell carcinoma and 3 were adenocarcinoma), the curative resection rate of ESD was 97% significantly higher than endoscopic mucosal resection cap-assisted (87%)[92]. However, EMR would be an alternative to lesions < 15 mm in diameter. One must note that ESD in the esophagus has been associated with perforation rates of 2% to 5% and stricture rates between 5% and 17.2%[90,93]. More data is needed to evaluate the utility of ESD for early esophageal adenocarcinoma in the United Stated.

Radiofrequency ablation

Radiofrequency ablation of BE with HGD is the most commonly used therapy, which has been shown to produce reproducible superficial injury in the esophagus (Figure 4). Its ease of use and better safety profile makes it a favorable therapy for flat lesions with HGD. The system generator is capable of delivering 10 to 12 J at a setting of 40 W/cm² with a depth of ablation between 500 and 1000 mm. Two delivery systems are currently available in use. A 3-cm-long balloon ablation catheter (HALO 360) intended to treat long-segment circumferential BE, and an endoscope-mounted targeted device (HALO 90) to treat short segments and tongues of BE. In a recent large series of 335 patients with BE and neoplasia (72% with HGD, 24% with IMC, 4% with low-grade dysplasia) in the United Kingdom who underwent RFA for BE-related neoplasia. The authors found that by 12 mo after

treatment, dysplasia was cleared from 81% of patients. Shorter segments of BE respond better to radiofrequency ablation (RFA)[94]. In another study of 70 patients who were treated. Seventy-four per cent had dysplasia (44 LGD, 8 HGD). Complete response was accomplished in 81% of patients[95]. A United Kingdom registry that follows the outcomes of 335 patients with BE who have undergone RFA for neoplasia and received endoscopic mucosal resection if nodules are found revealed HGD was cleared from 86% of patients, all dysplasia from 81%, and BE from 62% at the 12-mo time point, after a mean of 2.5 (range, 2-6) RFA procedures[94]. Of interest, endoscopic mucosal resection before RFA did not provide any benefit. Moreover, RFA appears to have a higher rate of complete histologic resolution response in comparison to photodynamic therapy (PDT) without any serious adverse events and was less costly than PDT for endoscopic treatment of Barrett's dysplasia[96]. Complications of RFA include chest and cervical pain, abdominal pain, dysphagia and stricture formation. Subsquamous neoplasia have been reported to develop after RFA for BE[97]. Currently, RFA is reserved for patients with BE with high-grade dysplasia with no visualized nodules. Its application for patients without dysplasia is debatable giving risks of complications and cost[98].

Photodynamic therapy

Photodynamic therapy has been used to photochemically eliminate abnormal mucosa. Porfimer sodium (POR) PDT use has been limited by serious side effects including prolonged cutaneous photosensitivity and stricture formation. In a randomized phase Ⅲ trial using POR and photodynamic therapy for ablating HGD in conjunction with omeprazole, POR PDT appears to be an effective therapy for ablating HGD in patients with BE and in reducing the incidence of esophageal adenocarcinoma[99]. PDT is associated with increased risks of stricture formation and of buried intestinal metaplasia or malignancy underneath neosquamous epithelium. In a study by Weiss *et al*[100] on 17 patients treated with PDT. High-grade dysplasia or early adenocarcinoma was completely eliminated in nine of 60% patients. Complications included stricture, sunburn, urticaria, small pleural effusions, esophageal spasm and transient atrial fibrillation. A recent randomized controlled trial of 5-Aminolaevulinic acid (ALA) *vs* Photofrin photodynamic therapy for high-grade dysplasia arising in BE showed no difference in complete reversal of HGD between the two groups. On sub-group analysis for BE ≤ 6 cm, complete reversal of HGD was significantly higher with ALA-PDT than Photofrin-PDT. Strictures and skin photosensitivity were significantly more common after treatment with Photofrin-PDT than ALA-PDT (33% *vs* 9% and 43% *vs* 6%, respectively, $P < 0.05$)[101].

Argon plasma coagulation

Argon plasma coagulation is a noncontact thermal tissue coagulation in which argon gas provides the medium for the delivery of an electric current[102]. This is accomplished with passing a probe through the working channel of the endoscope. The general setting for ablation of Barrett's mucosa is a high power setting 60-90 W at 1-2 L/min. Earlier study showed complete eradication of HGD and *in situ* adenocarcinoma was achieved after a mean number of 3.3+/-1.5 V. Argon plasma coagulation (APC) sessions in (80%)[103]. In a randomized controlled trial of 35 patients who received ablation of BE with multipolar electrocoagulation (16) *vs* argon plasma coagulation (19), the authors concluded complete reversal of BE can be maintained in approximately 70% of patients, irrespective of the technique[104]. Similarly, previous studies showed similar outcome with eradication of BE and restoration of squamous epithelium[105]. However, progression to HGD can still occur despite APC ablation[106]. Thus APC is effective ablative therapy for BE but the long term benefits are unknown. More data is needed on its use in early EAC.

Cryotherapy

Cryoablation is a relatively new technique with studies focusing on high-grade dysplasia and early-stage cancer in high-risk patients. It has an acceptable safety profile, and early results show response in a significant number of patients in whom other modalities have failed[107]. Its ease of use and lower chance of complication make it an attractive procedure. Although cryoablation is a non-tissue acquiring procedure that requires liquid nitrogen spray application it is not devoid of potential risk of gastric perforation due to gas insufflation. Data on its use in early EAC is limited. In a multicenter, retrospective cohort study of 79 patients with esophageal carcinoma in whom conventional therapy failed, refused and/or were ineligible for conventional therapy[108]. The study included all T staging and showed complete response of intraluminal disease in 31 of 49 subjects (61.2%), including 18 of 24 (75%) with mucosal cancer with an overall follow up of 10.6 months. No serious adverse events were reported. A recent study by Gosain *et al*[109] evaluated 32 patients with BE-HGD of any length who were treated with liquid nitrogen spray cryotherapy every 8 wk until complete eradication of HGD and intestinal metaplasia. Complete eradication of HGD achieved in 100% (32/32), and IM in 84% at 2-year follow-up. Recurrent HGD occurred in 18% with HGD. BE segment length ≥ 3 cm was associated with a higher recurrence of IM but not HGD. No serious adverse events occurred although stricture was seen in 9% of cases. Thus, cryoablation therapy appears comparable to other treating modality in BE and in early EAC, spray cryotherapy appears to have a unique role, eliminating mucosal cancer in 75% of patients[110].

A recent meta-analysis of seven studies involving 870 patients who underwent endotherapy (n = 510) or surgery (n = 360) concluded that endotherapy has similar efficacy to surgery but with lower adverse event rates. However, endotherapy was associated with a higher neoplasia recurrence rate[111]. Limitation to this study included small number of retrospective studies and different types

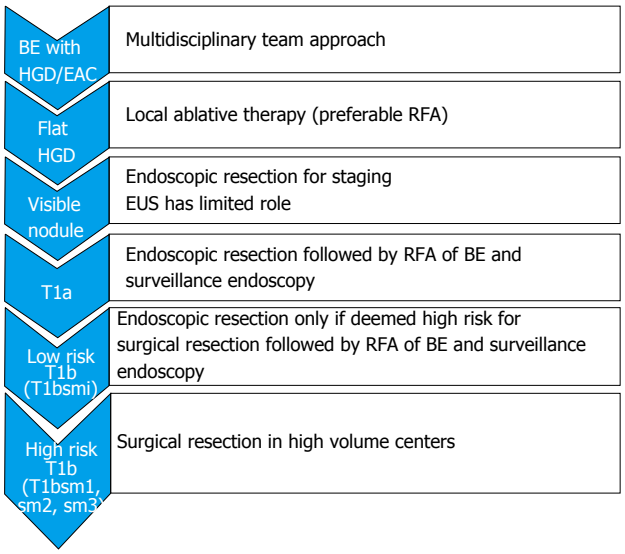

Figure 5 The current practical approach for patients with early esophageal neoplasia. BE: Barrett's esophagus; HGD: High grade dysplasia; EAC: Esophageal adenocarcinoma; EUS: Endoscopic ultrasound.

of endoscopic treatments used. Figure 5 shows the current practical approach to the management of patients with early EAC.

ROLE OF CHEMOPREVENTION

Esophageal adenocarcinoma is characterized by increasing incidence, male predominance and lack of preventive measures. Future preventive therapy might include the treatment of gastroesophageal acid reflux, obesity and/or chemoprevention with nonsteroidal antiinflammatory (NSAIDs) drugs or statins. Today, there is no evidence-based preventive measures are currently available for patients with EAC. Proton pump inhibitors are effective in reducing esophageal acid exposure and improve reflux symptoms however, they are not recommended for use as chemopreventive agents in EAC. Weight loss, exercise and bariatric surgery may potentially improve obesity. Studies have shown up-regulation of cyclooxygenase (COX)-2 in BE-metaplastic and dysplastic tissue and in Barrett's adenocarcinoma[112-114]. Others showed conflicting results[115]. NSAIDs and COX inhibitors have been proposed and shown to reduce risk of metaplasia in BE and EAC[116]. Statins have been suggested to induce anticancer effects against a variety of cancers in several studies[117]. Agents targeting the vascular endothelial growth factor and epidermal growth factor receptor pathways are currently in progress. The AGA recommendation for the chemoprevention of cancer in patients with BE is screening patients to identify cardiovascular risk factors for which aspirin therapy is indicated and against the use of aspirin solely to prevent esophageal adenocarcinoma in the absence of other indications[22].

CONCLUSION

Esophageal cancer is one of the most serious gastrointestinal cancers worldwide, owing to its rapid development and fatal prognoses in most cases. Major risk factors for EAC include BE, GERD, smoking, and obesity. Improved survival is achievable when the disease is confined to the more superficial mucosal layers and treated. Endoscopic luminal therapy is feasible and proven useful in BE with HGD and early esophageal adenocarcinoma.

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ORIGINAL ARTICLE

In vitro effects of polyphenols on colorectal cancer cells

Barbara Pampaloni, Gaia Palmini, Carmelo Mavilia, Roberto Zonefrati, Annalisa Tanini, Maria Luisa Brandi

Barbara Pampaloni, Gaia Palmini, Carmelo Mavilia, Roberto Zonefrati, Annalisa Tanini, Maria Luisa Brandi, Department of Surgery and Translational Medicine, University of Florence, Florence 50139, Italy

Author contributions: Pampaloni B and Mavilia C designed the study; Palmini G, Mavilia C and Zonefrati R performed the experiments; Pampaloni B and Palmini G wrote the manuscript; Tanini A and Brandi ML revised the manuscript; Brandi ML approved the final version of the manuscript.

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Correspondence to: Maria Luisa Brandi, MD, PhD, Department of Surgery and Translational Medicine, University of Florence, Largo Palagi 1, Florence 50139, Italy. marialuisa.brandi@unifi.it
Telephone: +39-55-7946304 Fax: +39-55-7946303

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Abstract

AIM: To investigate the effects of quercetin and genistein on colon cancer cell proliferation and their estrogen receptor β (ERβ) expression.

METHODS: Colon cancer cells were stably transfected with a mammalian expression vector to overexpress ERβ (HCT8-β8-expressing cells) or a control vector (HCT8-pSV2neo-expressing cells). The proliferation of these cells was examined after treatment with quercetin or genistein (5-100 μmol/L), or 10 nmol/L 17β-estradiol (17β-E2). Cell viability was examined by acridine orange staining following treatments for 48 or 144 h. Effects of quercetin and genistein on ERβ transcriptional transactivation were examined by luciferase activity in HCT8-β8-expressing cells transiently transfected with a pEREtkLUC reporter vector. In addition, the regulation of ERβ transcription by phytoestrogens and 17β-E2 was examined by quantitative polymerase chain reaction.

RESULTS: Proliferation of HCT8-β8-expressing cells was not reduced low doses (5 μmol/L) of quercetin and genistein, while it was reduced at 25-50 μmol/L with an effect similar to 10 nmol/L 17β-E2. Treatment with doses of phytoestrogens ⩾ 75 μmol/L completely blocked cell growth and reduced overall cell counts, however no effects at any dose were observed in HCT8-pSV2neo-expressing cells. These results were supported by viability staining that revealed acridine orange-stained lysosomes with high doses or extended treatment periods. Genistein and quercetin (50 μmol/L) significantly increased ER-responsive luciferase activity similar to 10 nmol/L 17β-E2 ($P < 0.05$). Furthermore, genistein and quercetin (50 μmol/L), as well as 10 nmol/L 17β-E2 significantly increased ERβ mRNA levels in HCT8-β8-expressing cells ($P < 0.05$). In addition, treatment of HCT8-pSV2neo-expressing cells with 50 μmol/L quercetin or 10 nmol/L 17β-E2 significantly increased ERβ mRNA levels compared to untreated controls ($P < 0.05$), though the absolute levels were much lower than in HCT8-β8-expressing cells.

CONCLUSION: The antitumorigenic effects of the phytoestrogenic compounds quercetin and genistein on colon cancers cells occur through ERβ activity and expression.

Key words: Estrogen receptor; HCT8-β8 cells; HCT8-pSV2neo; Quercetin; Genistein

Core tip: Colorectal cancer is one of the most common malignancies worldwide, though its incidence is lower in regions with a high dietary intake of estrogenic polyphenols. Moreover, the expression of estrogen receptor β (ERβ) is high in healthy colonic mucosa, and declines with the progression of colorectal cancer. This study examined the *in vitro* effects of two estrogenic polyphenols, quercetin and genistein, demonstrating their antiproliferative effects and regulation of ERβ activity and expression in colon cancer cells. These data suggest that a possible mechanism for the protective effects of such compounds is through activation and expression of ERβ.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies and a leading cause of cancer deaths for both men and women in Western countries[1]. The five-year survival rate remains poor despite significant advances in diagnosis and therapy. CRC results from an interaction among several factors, including lifestyle, family history and diet[2,3]. Since Lacassagne's work in 1955 demonstrating that estrogen administration increases the incidence of mammary cancer in mice[4], many studies have shown the involvement of sex hormones in the risk and development of many types of cancer, including breast cancer and CRC. The incidence of CRC is slightly lower in women compared to men of a similar age[5], and epidemiologic studies and results of the Women's Health Initiative clinical trial show that the risk is reduced in women who take hormone replacement therapy[6]. Furthermore, reduced serum levels of estradiol are associated with downregulated estrogen receptor (ER) expression in the colonic mucosa and a significantly increased risk of CRC[3,7].

ERα and ERβ are the two known subtypes through which estrogens exert their effects on various tissues. Experimental data show differential expression of these receptors, with very low levels of ERα either in normal or pathologic colonic mucosa (adenoma and carcinoma)[8], and high ERβ expression in healthy colonic mucosa, which decreases with the progression of CRC[8-11]. This has led to the proposal that ERβ functions as a tumor suppressor, protecting cells against malignant transformation, and is responsible for the protective effect of estradiol on CRC[12,13].

There is evidence that some polyphenols produced by plants have estrogen-like activity. It has been demonstrated that these phytoestrogens, with molecular structures similar to steroids, could be critical modulators of the human hormonal system and exert hormonal actions on target tissues[14,15]. Phytoestrogens have been widely studied for their potential therapeutic use in the prevention of different diseases and some carcinomas, given that they show some of the protective effects of estrogens in absence of the side effects associated with estrogen administration[16]. These effects may occur through binding to ERs or interacting with enzymes involved in sex steroid metabolism and biosynthesis[17]. Most phenolic compounds show a chemical structure similar to 17β-estradiol (17β-E2), suggesting they might compete for ER binding. However, phytoestrogens can produce estrogenic, anti-estrogenic and unique effects independent from estrogen binding recognition. These diverse actions of phenolic compounds are also tissue-specific, and thus are defined as selective estrogen receptor modulators[18].

Genistein is a phytoestrogen found in soy that may inhibit cancer progression by inducing apoptosis or inhibiting proliferation, the mechanisms by which are a subject of considerable interest[19]. A negative correlation was observed between the incidences of breast, prostate and colon cancer and the phytoestrogen-rich soy diet of some ethnic groups in Asia[20,21]. Recently, several studies have identified a dualistic mode of action by genistein in relation to cancer cell proliferation and cancer risk[22].

Whereas low concentrations of genistein have been shown to enhance the proliferation of breast cancer cells *in vitro*, high concentrations can inhibit their growth[23]. It is possible that the opposing effects of phytoestrogens depend on which ER isoform they interact with.

To better understand the influence of phytoestrogens on cancer development and progression, colon cancer cells were evaluated after exposure to genistein or quercetin, a flavonoid ubiquitously present in many fruits, vegetables, seeds, nuts, olive oil, tea and red wine[24] that also has potentially beneficial effects on cancer prevention[25-27]. The effect of these treatments on ERβ activation and expression, cell growth and cell viability, determined by staining with lysosomotropic acridine orange (AO) to detect lysosomal activation[28-30], were evaluated.

MATERIALS AND METHODS

Cell lines and chemicals

The human colon cancer HCT8 cell line[31,32] was obtained from the American Type Culture Collection (Rockville, MD, United States of America). Cells overexpressing human ERβ (HCT8-β8) were established *via* a stable transfection with the mammalian expression vector pCXN2-hERβ or a control pSV2neo vector (HCT8-pSV2neo)[33]. Genistein, quercetin and 17β-E2 (internal positive control) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Solutions of 17β-E2 and phytoestrogens were dissolved in ethanol and then diluted in cell culture medium to the final concentrations.

Cell culture

Cells were cultured in RPMI 1640 medium (Lonza Group, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS) or FBS-stripped serum (SFBS; Biological Industries, Kibhutz Beit Haemek, Israel), without phenol red, with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 100 μg/mL penicillin, 100 μg/mL streptomycin and 280.25 μg/mL Geneticin (G418; Invitrogen of Thermo Fisher Scientific Inc., Waltham, MA, United States) at 37 ℃ with 5% CO_2 humidified air. Confluent cell cultures were detached with a trypsin/ethylenediaminetetraacetic (EDTA) acid solution (Lonza Group) and plated at the desired density in the appropriate medium.

Cell proliferation analysis

For cell proliferation analysis, HCT8-β8- or HCT8-pSV2neo-expressing cells were plated on 6-well plates at a density of 5×10^3 cells/well. After 2 h, the medium was replaced with SFBS medium (phenol red-free medium supplemented with 10% SFBS, and penicillin-streptomycin) and stimulated with genistein or quercetin (5, 25, 50, 75, 100 μmol/L), or with 10 nmol/L 17β-E2 (cells without stimuli were used as a control). Cells were detached with trypsin/EDTA and the number was evaluated by a Bürker hemocytometer every 48 h for 8 d. Measurements for each dose at each time point were collected in triplicate and averaged.

AO staining

Following a 48 or 144 h treatment with quercetin, genistein or 17β-E2, HCT8-β8- or HCT8-pSV2neo-expressing cells were washed three times with phosphate buffered saline (PBS) to remove dead cells and serum proteins (cells without stimuli were used as a control). Cells were incubated in a 0.2% AO solution (in PBS, 2 mL/well) in the dark at room temperature for 10 min and washed three times with PBS. The cells were observed in phase contrast and under fluorescence (BP365/FT395/LP397 filter set) with an Axiovert 200 M microscope and images were acquired with Axiovision Software on an AxioCam HRC 12 megapixel camera (Carl Zeiss, Oberkochen, Germany). When stained with AO, DNA and mitochondria emit green fluorescence (530 nm) and lysosomes emit red fluorescence (650 nm) following excitation by ultraviolet (UV) light (365 nm).

Luciferase assay

HCT8-β8- or HCT8-pSV2neo-expressing were plated on 24-well plates at 2×10^4 cells/well in complete RPMI 1640 culture medium with 10% FBS and penicillin-streptomycin. Twenty-four hours later, the medium was replaced with phenol red-free medium supplemented with 10% SFBS and penicillin-streptomycin. A solution of Attractene Transfection Reagent (Qiagen, Venlo, Limburg, Netherlands) was used to transiently transfect cells with the pEREtkLUC (kindly supplied by Dr. MG Parker)[34] reporter plasmid (395 ng/well) and pERLNULL control plasmid (4 ng/well) (Promega, Madison, WI, United States), and cells were incubated in phenol- and FBS-free RPMI medium for 48 h. After a 24 h stimulation in the same medium with quercetin (50 μmol/L), genistein (50 μmol/L) or 17β-E2 (10 nmol/L) (or no stimulation for controls), whole cell extracts were obtained with the Luciferase Assay System (Promega) and luciferase activity was determined with a luminometer (LKB Instruments, Mount Waverly, Victoria, Australia). Luciferase activity was normalized to β-galactosidase activity measured by a β-gal Assay Kit (Invitrogen) and to total protein concentration. Measurements for each condition were collected in triplicate and averaged.

RNA isolation and real-time quantitative polymerase chain reaction

Total RNA was isolated from cultured cells after stimulation with quercetin (50 μmol/L), genistein (50 μmol/L) or 17β-E2 (10 nmol/L) (from triplicate plates) with TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified by UV absorbance. Reverse transcription was performed using the Quantitect Reverse Transcription Kit followed by treatment with ribonuclease-free deoxyribonuclease I (Qiagen). Quantitative polymerase chain reaction (qPCR) was performed using the Kapa Probe Fast qPCR kit (Kapa Biosystems Inc., Wilmington, MA, United States) according to the manufacturer's instructions. Briefly, reactions consisting of 2 μL cDNA, 10 μL KAPA PROBE FAST qPCR Master Mix, 2 μL gene specific primers (10 μmol/L), 1 μL TaqMan Probe (5 μmol/L), and 5 μL RNase-free H_2O were heated at 95 ℃ for 5 min and amplified by 35 cycles of 95 ℃ for 10 s, and 60 ℃ for 30 s using a Rotor-Gene Q (Qiagen). The results obtained were normalized to a housekeeping gene (*RPS18*).

The following primers and corresponding TaqMan probes were used: ERβ: (forward) 5'-TCGCCAGTTATCACATCTGTATGCGG-3', (reverse) 5'-GTGTCTCTCTGTTTACAGGTAAGGTGTG-3', (probe) F/TCCCTGGTG/ZEN/TGAAGCAAGATCGCTAGAA/Q; RSP18: (forward) 5'-CTTCCACAGGAGGCCTAC-3', (reverse) 5'-GATGGCAAAGGCTATTTTCCG-3', (probe) F/TTCAGGGAT/ZEN/CACTAGAGACATGGCTGC/Q.

Statistical analysis

Statistical differences between groups were analyzed in Microsoft Excel (Microsoft, Redmond, WA, United States) using Student's *t*-tests. Data are expressed as mean ± SD. Statistical differences for cell proliferation analysis between treated groups *vs* controls were analyzed in Excel using a parallelism test for linear regression.

RESULTS

Effects of genistein and quercetin on colon cancer cell proliferation

Cell counts of HCT8-β8- or HCT8-pSV2neo-expressing cells cultured with genistein, quercetin or 17β-E2 were performed every 48 h for up to 12 d to assess cell proliferation. Results show that both phytoestrogens dose-dependently significantly reduced the proliferation of HCT8-β8-expressing cells (Figure 1A and B). The inhibition of cell growth by genistein and quercetin was apparent at concentrations of 25 μmol/L, similar to the effects 10 nmol/L 17β-E2. However, higher concentrations of the phytoestrogens (75 and 100 μmol/L) prevented proliferation and reduced overall cell counts. In contrast, quercetin, genistein and 17β-E2 treatments had no effect on the proliferation of HCT8-pSV2neo-expressing cells (Figure 1C and D).

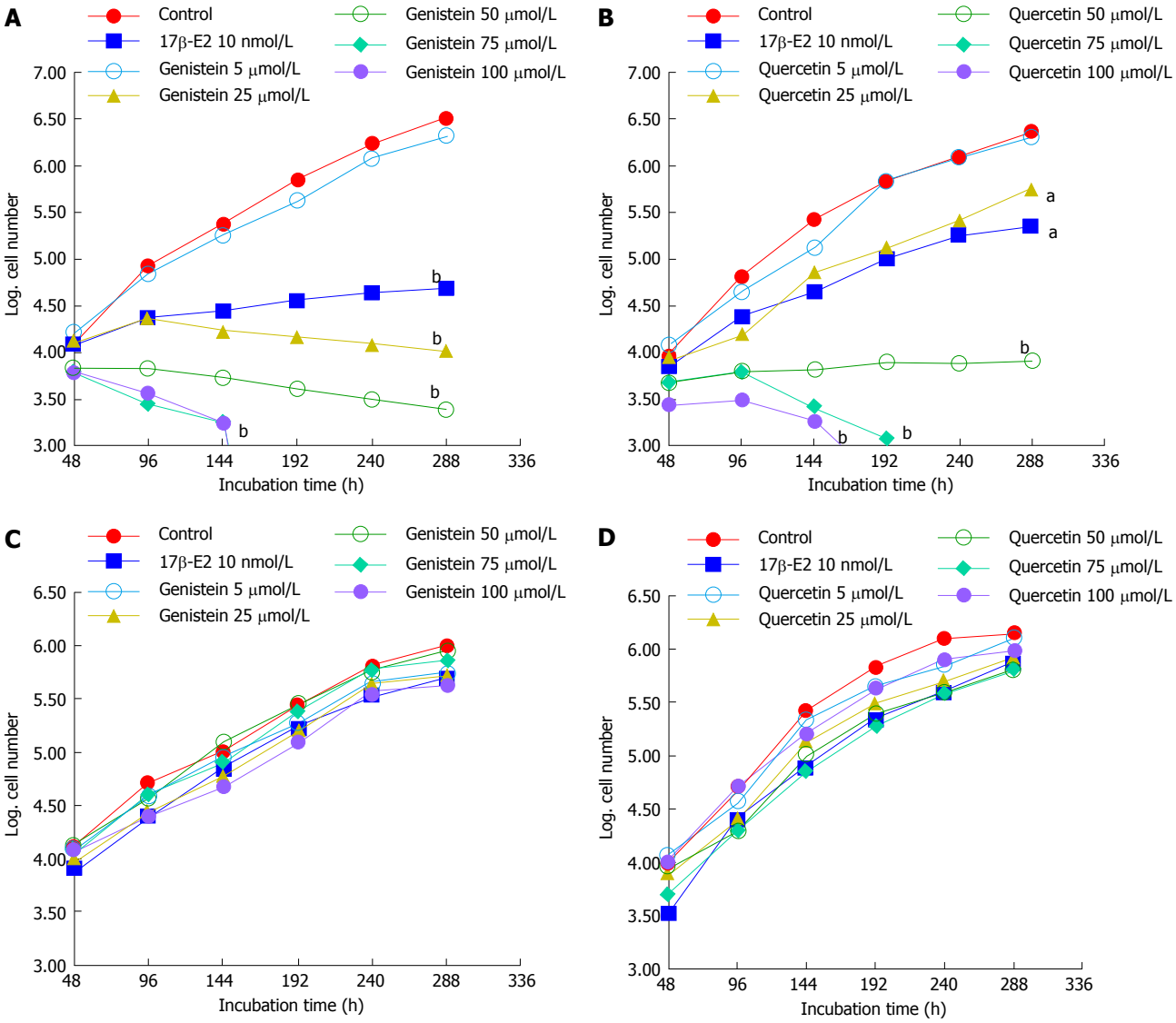

Figure 1 Effects of polyphenols on cell growth. A: Growth of HCT8-â8-expressing cells in the presence of genistein and 17â-E2; B: Growth of HCT8-β8-expressing cells in the presence of quercetin and 17β-E2; C: Growth of HCT8-pSV2neo-expressing cells in the presence of genistein and 17β-E2; D: Growth of HCT8-pSV2neo-expressing cells in the presence of quercetin and 17β-E2. Values are the means of triplicates; [a]$P < 0.05$ *vs* control; [b]$P < 0.01$ *vs* control.

Effects of genistein and quercetin on colon cancer cell viability

AO staining of HCT8-β8-expressing cells treated for 48 h with 5-25 μmol/L genistein (Figure 2B and C), 5-25 μmol/L quercetin (Figure 3B and C) or 10 nmol/L 17β-E2 (Figure 4B) revealed a homogenous green brilliant fluorescence, similar to the untreated control cells. However, red lysosomes became apparent with higher doses of both phytoestrogens (≥ 50 μmol/L) (Figures 2D-F, 3D-F), or extended exposure of concentrations ≥ 25 μmol/L (144 h; Figures 2I-L, 3I-L). There were some red-labeled lysosomes observed with 144-h treatment of 10 nmol/L of 17β-E2 (Figure 4D). Long-term treatment with high doses of phytoestrogens (≥ 75 μmol/L) revealed many cells with pale and homogeneous green fluorescence and many brilliant red-orange lysosomes (Figures 2K, L, and 3K, L), which indicate reduced viability and cellular stress. In contrast, HCT8-pSV2neo-expressing cells were largely unaffected by treatment with genistein (Figure 5), quercetin (Figure 6B), or 17β-E2 (Figure 4E-H), but rather exhibited strong, homogeneous green fluorescence with few lysosomes in all the treated samples after 48 and 144 h.

Effects of genistein and quercetin on ERβ transactivation

To determine if the anti-proliferative effects of genistein and quercetin occurred through activation of ERβ, ER-responsive luciferase activity was measured in HCT8-β8-expressing cells transiently transfected with the pEREtkLUC reporter plasmid. Luciferase activity was significantly increased (165%) following 24 h treatment with 10 nmol/L 17β-E2 ($P < 0.05$) (Figure 7). Similarly, treatment with 50 μmol/L genistein and 50 μmol/L quercetin produced an increase in luciferase activity of 158 and 81%, respectively ($P < 0.05$), compared to an un-

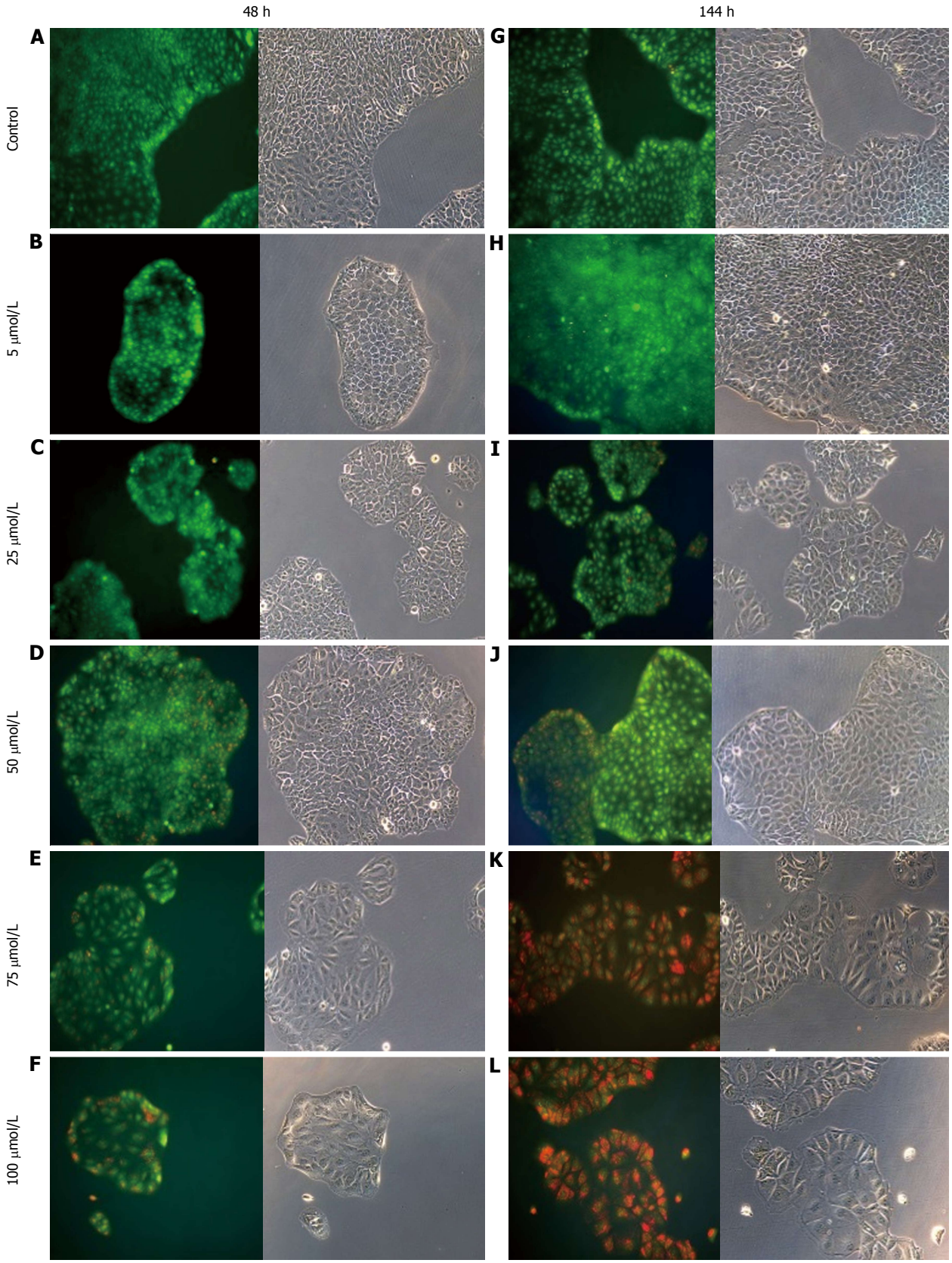

Figure 2 Treatment of HCT8-β8-expressing cells with genistein. HCT8-β8-expressing cells were treated with various concentrations of genistein for 48 h (A-F) or 144 h (G-L) and stained with acridine orange. Nuclei and mitochondria appear green, whereas lysosomes appear red-orange under fluorescence, adjacent to corresponding phase contrast images (magnification × 20).

treated control. ER-responsive luciferase activity was not evaluated for HCT8-pSV2neo-expressing cells as neither of the two polyphenols produced anti-proliferative effects in this cell line.

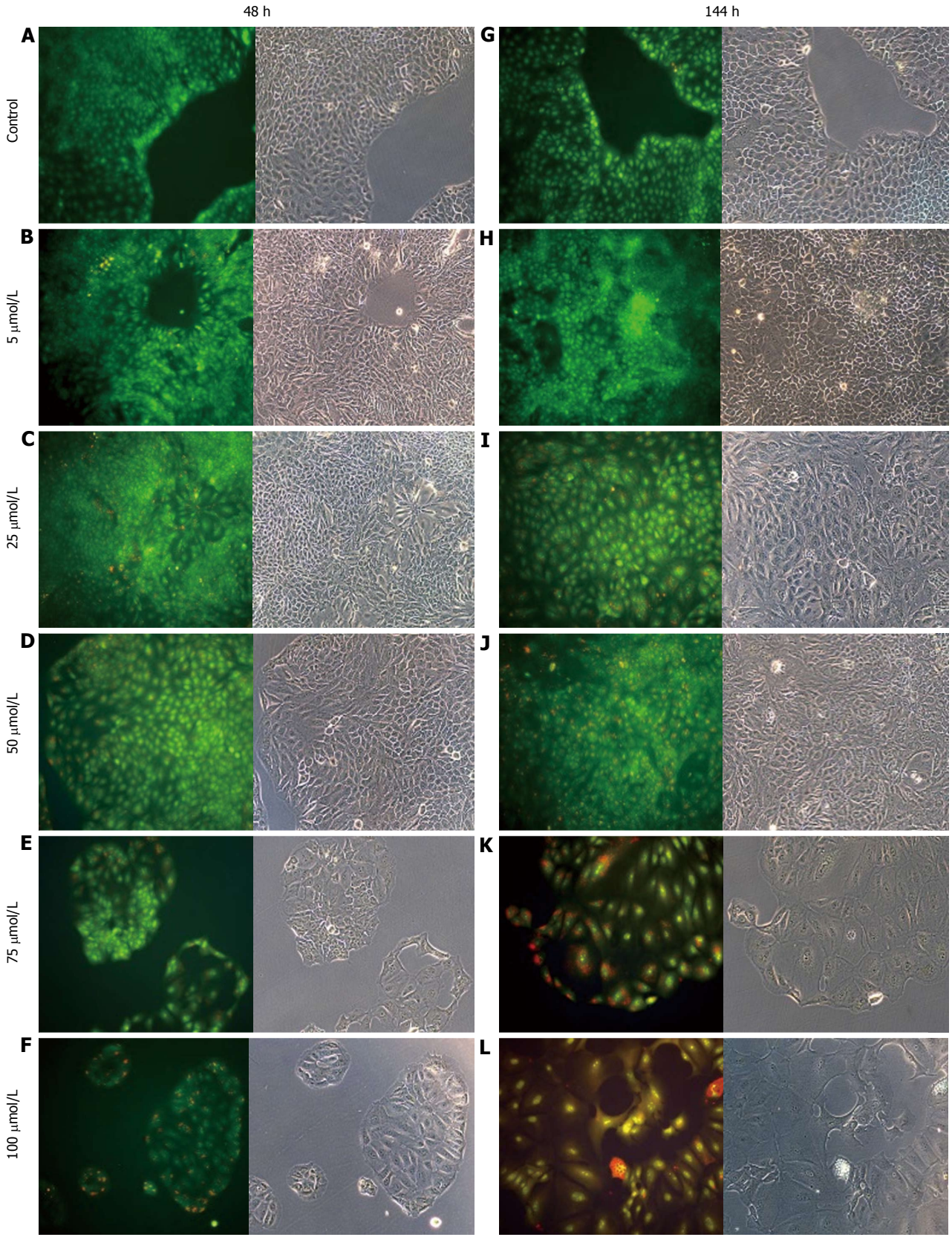

Figure 3 Treatment of HCT8-β8-expressing cells with quercetin. HCT8- β8-expressing cells were treated with various concentrations of quercetin for 48 h (A-F) or 144 h (G-L) and stained with acridine orange. Nuclei and mitochondria appear green, whereas lysosomes appear red-orange under fluorescence, adjacent to corresponding phase contrast images (magnification, × 20).

Effects of genistein and quercetin on ERβ transcription

The expression of ERβ mRNA in HCT8-β8-expressing cells was significantly increased following a six-day treatment with 50 μmol/L genistein ($1.39 \times 10^8 \pm 5.33 \times$

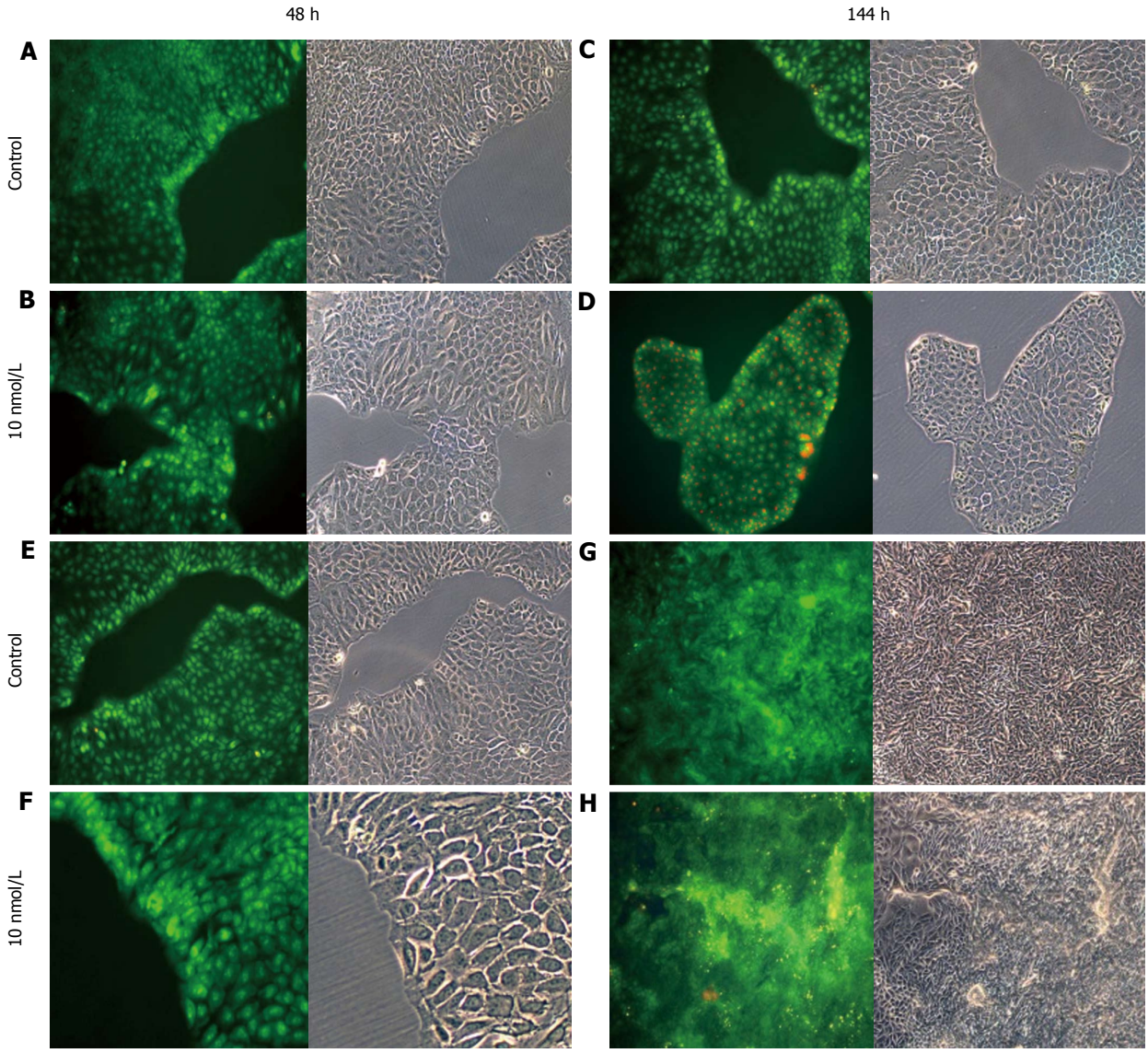

Figure 4 Treatment of cells with 17β-E2. A-D: HCT8-β8-expressing cells; or E-H: HCT8-pSV2neo-expressing cells were treated with 10 nmol/L 17β-E2 for 48 h (A, B, E, F) or 144 h (C, D, G, H) and stained with acridine orange. Nuclei and mitochondria appear green, whereas lysosomes appear red-orange under fluorescence, adjacent to corresponding phase contrast images (magnification × 20).

10^7), 50 μmol/L quercetin ($1.45 \times 10^8 \pm 5.00 \times 10^7$) and 10 nmol/L 17β-E2 ($1.49 \times 10^8 \pm 4.35 \times 10^7$), compared to untreated controls ($5.00 \times 10^7 \pm 1.90 \times 10^7$) (all $P < 0.05$) (Figure 8A). Increases in ERβ mRNA levels were also observed in HCT8-pSV2neo-expressing cells treated with quercetin ($5.88 \times 10^6 \pm 3.20 \times 10^6$) and 17β-E2 ($1.91 \times 10^6 \pm 8.54 \times 10^5$) ($P < 0.05$) (Figure 8B), though the relative expression ($3.97 \times 10^5 \pm 1.37 \times 10^5$) was much lower compared to HCT8-β8-expressing cells.

DISCUSSION

Genistein, found in soybeans and their derivatives, and quercetin, one of the most abundant phytoestrogens in the Western diet[34], are two natural flavonoid molecules with molecular structures similar to 17β-E2, which is a substrate of ERβ. Consumption of phytoestrogen-rich foods is correlated with a reduced incidence of CRC[35,36]. Moreover, plasma concentrations of phytoestrogens are high in populations from China, Japan and countries of Southeast Asia, which are considered to have low risks for malignancy, particularly for hormone-sensitive cancers such as breast cancer, prostate cancer and CRC[20,37,38].

The possible antitumorogenic effects of phytoestrogens were tested in two CRC cell models, including a hormone-sensitive cell line of colon adenocarcinoma expressing very low levels of ERβ (HCT8-pSV2neo-expressing), and the same cell line with high levels of ERβ (HCT8-β8-expressing). The range of phytoestrogen concentrations used were based on epidemiologic and absorption human studies. Quercetin intake is reported to be approximately 16 mg/d[34], and a study by Hollman *et al*[39] found that 76% of orally administered quercetin aglycone is recovered in the ileostomy bags of subjects who underwent a colectomy, which can be considered a model compartment for the colon[40]. Therefore, an aver-

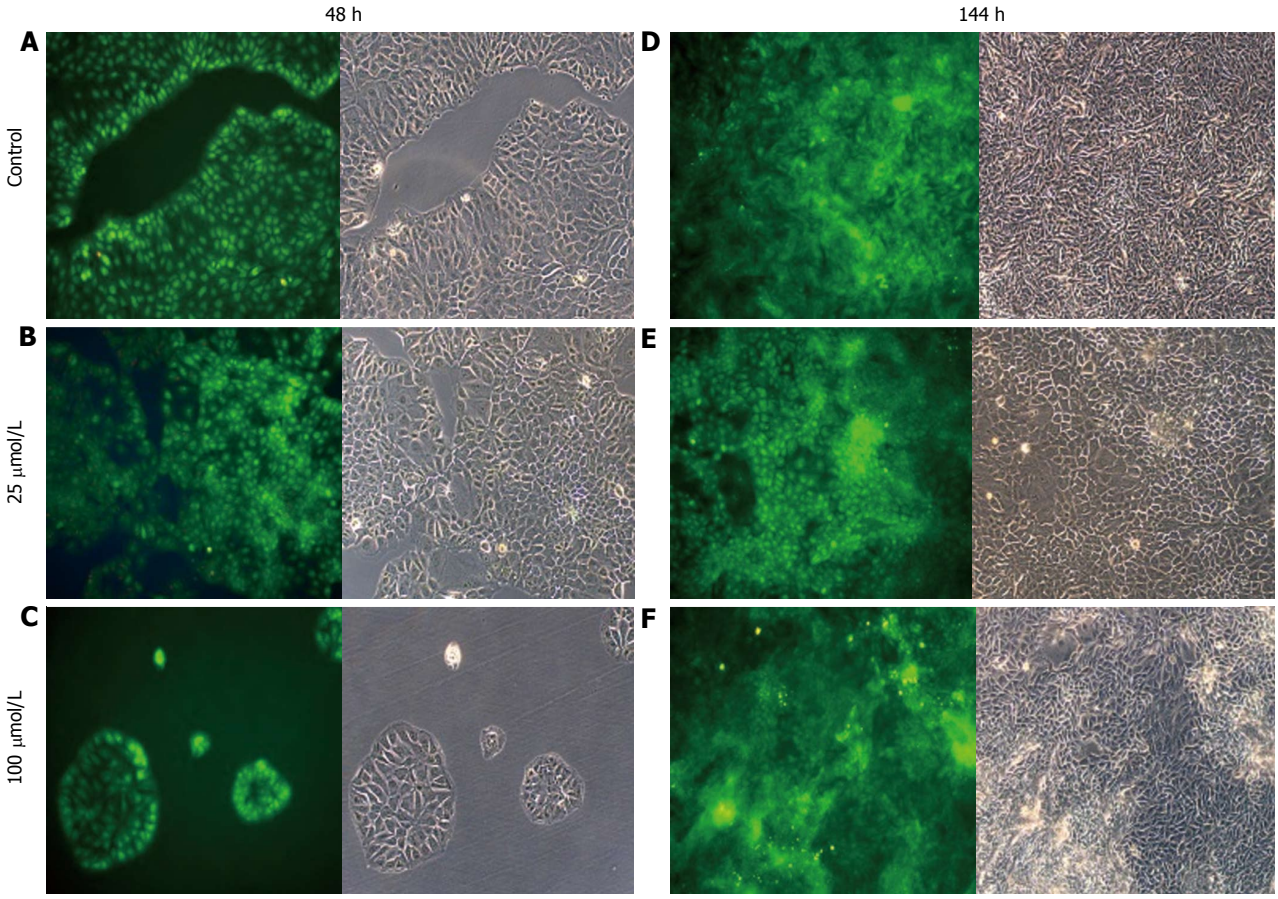

Figure 5 Treatment of HCT8-pSV2neo-expressing cells with genistein. A-F: HCT8-pSV2neo-expressing cells were treated with 25 μmol/L (B and E) or 100 μmol/L (C and F) genistein for 48 h (A-C) or 144 h (D-F) and stained with acridine orange. Nuclei and mitochondria appear green, whereas lysosomes appear red-orange under fluorescence, adjacent to corresponding phase contrast images (magnification × 20).

age 12 mg of quercetin reaches the colon daily, indicating that, depending on dietary intake, quercetin concentrations of 40-80 μmol/L in the colon are likely.

Dietary intakes of 39 and 47 mg of genistein/day for the adult Chinese and Japanese populations, respectively, have been reported[41-43], whereas the Western diet provides only 1-2 mg/d, with values of up to 3-12 mg of genistein/day for those following a vegetarian diet[44,45].

The results of the *in vitro* proliferation analyses show that even relatively low doses of phytoestrogens can reduce, and concentrations comparable to those found in Eastern diets can block, proliferation of HCT8-β8-expressing, but not HCT8-pSV2neo-expressing cancer cells. These data confirm results described in the literature regarding the behavior of the same phytoestrogens on different CRC cell lines, as well as in other hormone-sensitive cancer cells[34,46-48]. For example, genistein has an anti-proliferative effect on the estrogen-dependent human breast cancer MCF-7 cell line similar to that induced by 17β-E2[23], and the proliferation of prostate cancer cells is reduced by quercetin[24]. However, a study on the Caco-2 colon cancer cell line, which contains low levels of ERβ, showed that cell cycle gene expression and cell proliferation was reduced with 50 μmol/L of quercetin, resulting in cell cycle arrest[25,26].

The observed anti-proliferative effects of phytoestrogens on HCT8-β8-expressing cells were accompanied by activation of ERβ, as observed by luciferase activation. The results show that both genistein and quercetin increased luciferase activity, comparable to levels induced by 17β-E2. This activity likely depends directly on ERβ binding, which can then modulatate the expression of specific proteins directly involved in cell cycle regulation[49-55]. Furthermore, the concentrations of quercetin and genistein that inhibited cell growth but did not induce cell death were also found to increase ERβ mRNA levels. The basal level of ERβ in HCT8-β8-expressing cells perpetuated a large increase in mRNA after treatment with both phytoestrogens and 17β-E2. A proportionately larger increase was observed in HCT8-pSV2-neo-expressing cells, though the relative levels were much lower.

Taken together, these data suggest that the inhibition of cell growth, activation of ERβ and the increased transcription of ERβ depend on the binding of phytoestrogens to ERβ, as these effects were absent or minimal in HCT8-pSV2neo-expressing cells, though future experiments with agents blocking the estrogen receptor will be necessary to confirm this. The data presented here are in agreement with observations from other hormone-

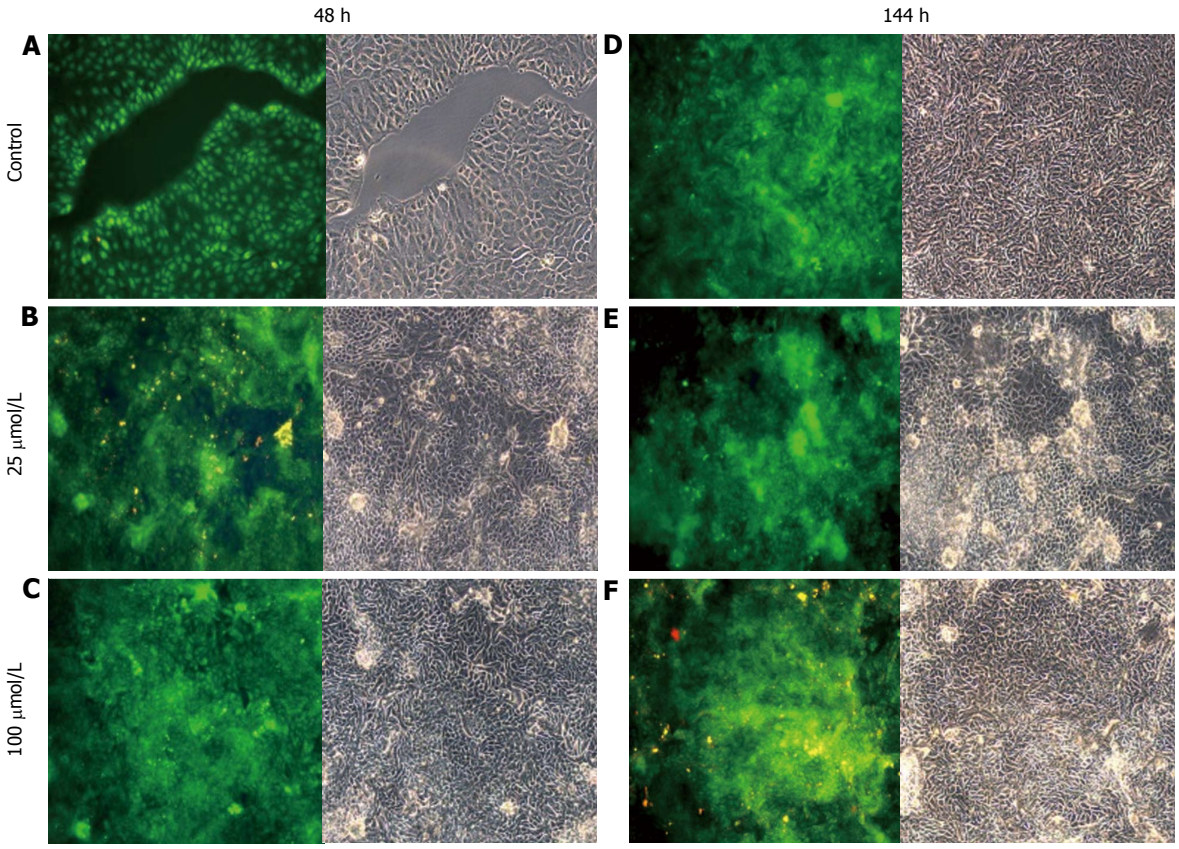

Figure 6 Treatment of HCT8-pSV2neo-expressing cells with quercetin. A-F: HCT8-pSV2neo-expressing cells were treated with 25 μmol/L (B and E) or 100 μmol/L (C and F) quercetin for 48 h (A-C) or 144 h (D-F) and stained with acridine orange. Nuclei and mitochondria appear green, whereas lysosomes appear red-orange under fluorescence, adjacent to corresponding phase contrast images (magnification × 20).

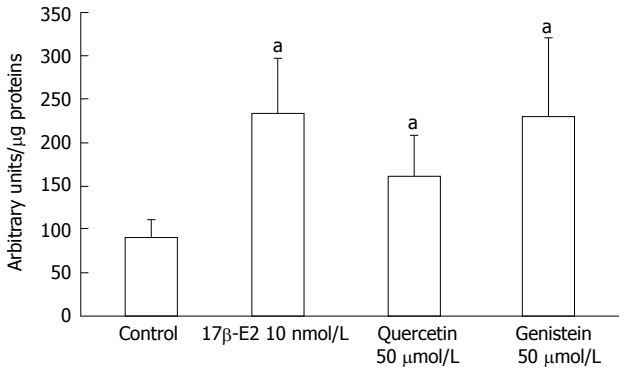

Figure 7 Induction of EREtkLUC reporter gene activity. Treatment of HCT8-â8-expressing cells with 17â-E2, genistein and quercetin induces EREtk expression observed as relative luciferase activity. Values are the mean ± SD of triplicates; $^{a}P < 0.05$ *vs* control.

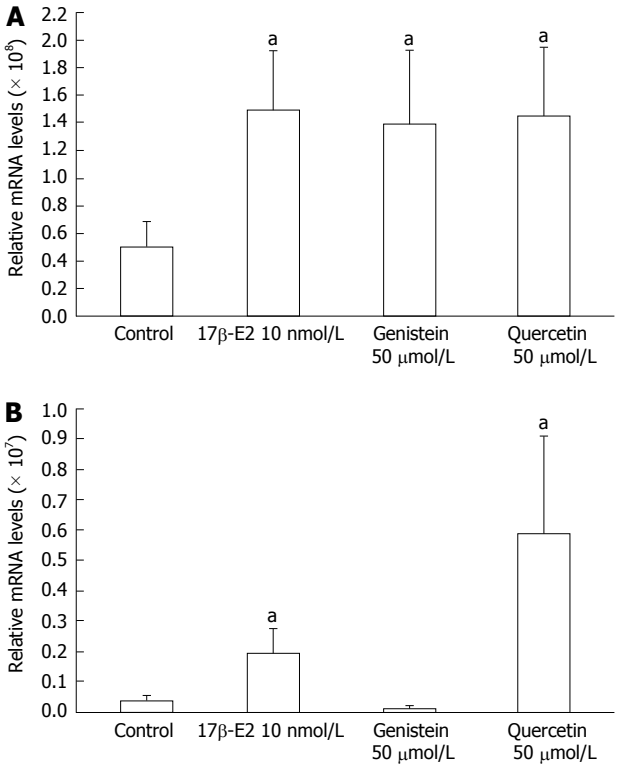

Figure 8 Expression of ERβ mRNA levels by quantitative real-time reverse transcription-polymerase chain reaction. Induction of ERβ expression by 17β-E2, genistein and quercetin in A: HCT8-β8-expressing cells; B: HCT8-pSV2neo-expressing cells. The results are expressed relative to RPS18 mRNA levels. Values are the mean ± SD of quadruplicates; $^{a}P < 0.05$ *vs* control.

sensitive cancers[25,56], and also demonstrate the protective role of ERβ that has been reported for estrogen-sensitive tissue such as breast, ovary, prostate and colorectal mucosa[57-61]. Furthermore, these results support the epidemiologic and experimental data which show the protective action of both the tested phytoestrogens at a concentration similar to the levels in colorectal mucosae that result from daily phytoestrogen intake in the Eastern diet, and indicate that dietary intake of phytoestrogens may protect against CRC by acting on tumoral cell growth and modulating gene transcription. In conclusion, our study indicates that the mechanism for antitumorogenic activity

of phytoestrogens on CRC could involve regulation of ERβ expression.

COMMENTS

Background

Recent evidence suggests a close relationship between estrogen and colorectal cancer (CRC), one of the most common malignancies, such that reduction in circulating levels of estradiol increases the risk of developing cancer. Furthermore, regions with a high dietary intake of phytoestrogens, natural molecules with estrogen-like effects, have lower incidences of CRC. The expression of estrogen receptor β (ERβ), is high in healthy colorectal mucosa, and reduced in cancerous tissue. However, the mechanism regulating the effect of estrogen on the development of CRC is not well understood.

Research frontiers

Among the phytoestrogens examined for their antitumoral functions, the flavonoids genistein and quercetin are the most well studied. In this *in vitro* study, the authors evaluate these two phytoestrogens, which are common in food sources, and suggest that their anti-proliferative effects are through the activation and expression of ERβ.

Innovations and breakthroughs

Several *in vivo* studies have highlighted the protective antitumoral role of two phytoestrogens, quercetin and genistein, in different hormone-sensitive cancers and the protective role of ERβ on estrogen-sensitive tissues such as breast, ovary, prostate and colorectal mucosa. This *in vitro* study confirms epidemiologic and experimental data which show the protective action of these phytoestrogens against CRC, and demonstrate their effect on cancer cell growth and ERβ transcription. In particular, this study reveals that these effects occur at concentrations of quercetin that are equivalent to those obtained following a daily intake of 16 mg/d.

Applications

By studying the influence of phytoestrogens on the growth of colon cancer cells and their regulation of ERβ expression, this study suggests that similar results could also be found for other hormone-sensitive tissues. Furthermore, the results further suggest that an increase in the dietary consumption of foods rich in phytoestrogens could represent a future strategy for the prevention of CRC and other hormone-sensitive cancers.

Terminology

Estrogen receptors ERα and ERβ are activated by 17-estradiol. Phytoestrogens are a group of plant-derived compounds, including flavonoids, coumestans, lignans and stilbenes, with estrogenic properties. Genistein and quercetin are the most representative of the phytoestrogens that have been studied for their antitumorigenic properties.

Peer review

This study examines the biologic effects of two phytoestrogens on cell growth and expression of ERβ in colon cancer cell lines. The results indicate that quercetin and genistein exert their effects by activating and regulating the expression of ERβ. This study has significance for guiding future preventive therapies for colorectal cancer.

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CASE REPORT

Neuroendocrine tumors of the gastrointestinal tract: Case reports and literature review

William J Salyers, Kenneth J Vega, Juan Carlos Munoz, Bruce W Trotman, Silvio S Tanev

William J Salyers, Kenneth J Vega, Juan Carlos Munoz, Division of Gastroenterology, University of Florida College of Medicine/Jacksonville, Jacksonville, FL 32207, United States
Kenneth J Vega, Division of Digestive Diseases and Nutrition, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States
Bruce W Trotman, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, United States
Silvio S Tanev, Department of Pathology, Manatee Memorial Hospital, Bradenton, FL 34208, United States
Author contributions: Salyers WJ collected the data at the U of Florida College of Medicine/Jacksonville and wrote the initial manuscript; Vega KJ and Munoz JC supervised the initial manuscript; Trotman BW contributed cases and edited the manuscript; Tanev SS gathered the pathology slides and prepared the slides for publication.
Correspondence to: Kenneth J Vega, MD, Division of Digestive Diseases and Nutrition, University of Oklahoma Health Sciences Center, 920 Stanton L Young Boulevard, WP 1345, Oklahoma City, OK 73104,
United States. kenneth-vega@ouhsc.edu
Telephone: +1-405-2715428 Fax: +1-405-2715803
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Abstract

Neuroendocrine tumors (NET) previously called carcinoid tumors are neoplasms of enterochromaffin/neuroendocrine cell origin which display neurosecretory capacity that may result in the carcinoid syndrome. The annual incidence of patients with NET is 8.4 per 100000; yet many NET remain asymptomatic and clinically undetected. A majority of NET follows a benign course; however, some will display malignant characteristics. NET most commonly occur in the gastrointestinal tract (67%) and bronchopulmonary system (25%). Gastrointestinal NET occur within the stomach, small intestine, liver, and rectum. We report a retrospective study of 11 subjects: Eight with benign carcinoid tumors: duodenal bulb (*n* = 2), terminal ileum (*n* = 1), sigmoid colon (*n* = 2), and rectum (*n* = 3); three with malignant carcinoid: liver (*n* = 1) and intra-abdominal site (*n* = 2). The diagnosis, endoscopic images, outcome, treatment and review of the literature are presented.

Key words: Neuroendocrine; Carcinoid; Gastrointestinal; Tumors

Core tip: Endoscopic procedures sometimes reveal submucosal lesions within the gastrointestinal tract that are resected and confirmed as neuroendocrine tumors by appropriate immunochemical stains. Most will be benign as demonstrated in our series of 11 subjects. This case series of gastrointestinal neuroendocrine tumors reminds every endoscopist to carefully examine the upper and lower gastrointestinal tract for such lesions.

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INTRODUCTION

Historically described as a more indolent behaving tumor than adenocarcinoma by Oberndorfer in Germany in 1907, neuroendocrine (carcinoid) tumors (NET) are undergoing a location change within the gastrointestinal tract[1-4]. A shift in the anatomic location has occurred over the last half-century. Data from 1950 to 1971 identified the appendix as the most common site followed by rectal and ileum for NET[4]. However, a recent evaluation of carcinoid tumors identified in the Surveillance, Epidemiology and End Results Program between 1973

Table 1 Clinical data of patients with neuroendocrine tumors

Patient (age, yr/sex)	Initial evaluation	Site	Diagnostic studies	Outcome
65/F	Hematochezia, IBD epigastric pain	Duodenal bulb	12 21 05 EGD duodenal bulb polyp; path: neuroendocrine tumor 12 30 05 repeat EGD, no residual, path: neuroendocrine tumor 11 24 08 repeat EGD no recurrence, COL mucosal prolapse syndrome	Alive and well
59/M	GERD with break-through symptoms	Duodenal bulb	11 11 08 EGD duodenal bulb polyp, path: neuroendocrine tumor 12 22 08 EGD, no residual tumor 12 30 08 PET scan negative	Alive and well
50/F	2nd opinion for liver metastatic disease	Liver	02 09 04 EGD chronic esophagitis, HH, fundic nodularity, path: benign lymphoid aggregates 03 16 04 PET/CT innumberable larg hepatic lesions replacing R and L lobes consistent with neuroendocrine tumor	Expired 12 04
70/M	Epigastric pain and 15 lb weight loss	Intra-abdominal	04 15 08 EGD chronic esophagitis, HH, acute and chronic gastritis; path: reactive gastropathy; COL: 1 adenomatous/2 hyperplastic polyps 04 16 08 CT Abd/Pelvis mesen-teric mass 04 24 08 CT guided bx: path: neuroendocrine tumor	05 08 treated with sandostatin
46/F	Nausea, vomiting, abdominal pain	Intra-abdominal	01 02 10 CT Abd/Pelvis ascites small bowel and colonic obstruction 01 04 10 Gastrografin emema sigmoid Obstruction 01 04 10 exploratory laparotomy desmoplastic reaction, sigmoid colon with liver metastases and intraperitoneal implants; bx of implants positive for chromogranin and synapotophysin 01 19 10 COL 3 cm stenosis at 30 cm due to extrinsic pressure; stent placed 01 26 10 serum CGA, 27 nmole/L	Discharge To hospice
40/M	Recurrent perianal abscess r/o IBD	Terminal ileum	12 05 06 COL 10 mm sessile polyp in terminal ileum, path: neuroendocrine tumor	Lost to follow-up
50/F	GERD and CRCS	Sigmoid	04 04 08 EGD chronic esophagitis, HH, path: mild reactive gastropathy, COL 4 mm sigmoid neuroendocrine tumor resected 04 30 08 normal octreotide scan 03 30 09 COL negative bx at prior polypectomy site	Alive and well
75/F	Breast cancer and CRCS	Sigmoid	02 06 08 COL 7 mm sigmoid submucosal nodule resected; cells positive for synaptophysin, but negative for chromogranin 03 11 08 Urinary 5-HIAA negative 04 22 08 Repeat COL with resection of remaining neuroendocrine tumor 05 19 09 COL negative for recurrence	Alive and well
55/M	LLQ tenderness, CRCS	Rectum	08 22 06 COL sigmoid tubulovillous adenoma and 6 mm rectal neuro-endocrine tumor 09 01 09 COL hyperplastic polyp, no recurrence of neuroendocrine tumor	Alive and well
55/F	CRCS	Rectum	05 01 09 COL 8 mm neuroendocrine tumor COL 1 yr later no recurrence	Alive and well
60/F	CRCS	Rectum	11 29 07 COL submucosal nodule neuroendocrine tumor 01 28 08 COL no recurrence	Alive and well

IBD: Inflammatory bowel disease; CGA: Chromogranin A; EGD: Esophagoduodenoscopy; COL: Colonoscopy; GERD: Gastroesophageal reflux disease; HH: Hiatal hernia; R: Right; L: Left; bx: Biopsy; CRCS: Colorectal cancer screening; F: Female; M: Male; 5-HIAA: 5-hydroxyindoleacetic acid; PET/CT: Positron emission tomography/computed tomography; LLQ: Left lower quadrant.

and 1999 found the ileum to be the most frequent site of gastrointestinal NET followed by the rectum; the appendix accounted for only 4.8% of NET[4]. Additionally, gastric NET accounted for an increasing proportion of gastrointestinal NET[4,5]. This change in location of NET has resulted from changes in diagnostic modalities used as well as reporting techniques over time[6]. The estimated incidence in the United States ranges from 2.5-5 cases per 100000[4]. A European investigation which included both surgical and autopsy specimens, reported an overall incidence of 8.4 cases per 100000[4,7,8]. Incidence estimates are limited by the clinically silent nature of many NET which remain undetected until autopsy[6].

CASE REPORT

This case series describes a wide spectrum of benign gastrointestinal NET originating in the small intestine (n = 2), terminal ileum (n = 1), colon (n = 2), rectum (n = 3), malignant NET of the liver (n = 1) and intraabdominal sites (n = 2) (Table 1).

Patient 1

A 65-year-old female with a history of possible inflam-

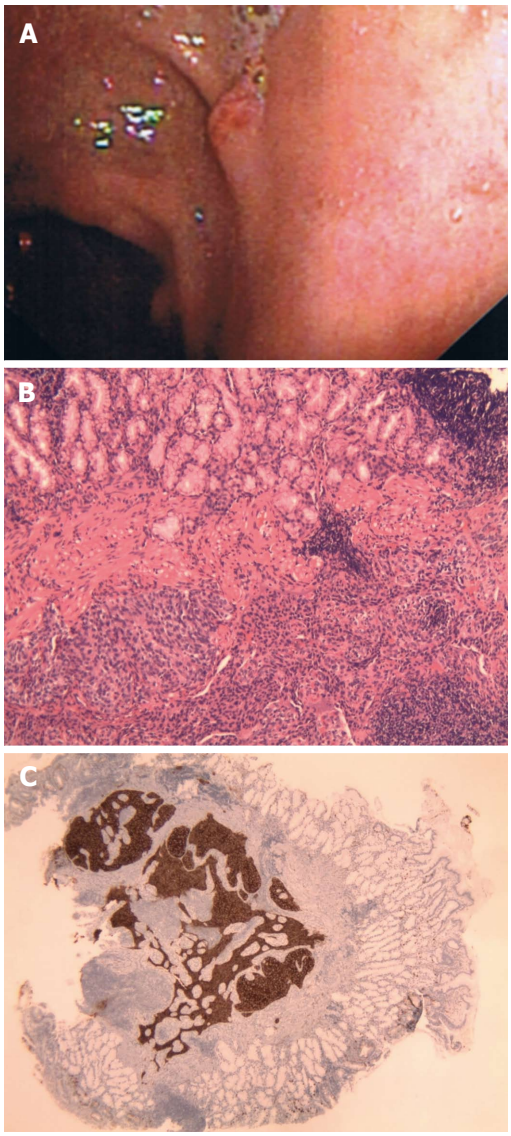

Figure 1 A 65-year-old female with a history of possible inflammatory bowel disease presented for evaluation of epigastric pain and occasional hematochezia. A: Patient 1, neuroendocrine (carcinoid) tumors as duodenal nodule at endoscopy; B: Solid growth pattern with organoid architecture and bland monotonous cells with lack of significant atypia and increased mitoses. H and E, × 10; C: Neoplastic neuroendocrine cells show diffuse positivity for Chromogranin. Chromogranin, × 20.

matory bowel disease presented for evaluation of epigastric pain and occasional hematochezia. Colonoscopy revealed multiple polypoid lesions throughout the colon with biopsies consistent with mucosal prolapse syndrome. Esophagogastrastroduodenoscopy (EGD) revealed mild esophagitis, chronic gastritis, and a 5 mm polyp in the duodenal bulb biopsied with cold forceps (Figure 1A). Pathology demonstrated duodenal mucosa with atypical organized nests of cells with expression of low molecular cytokeratin, neuron-specific enolase (NSE), chromogranin, and synaptophysin on immunohistochemistry consistent with a neuroendocrine tumor (Figure 1B and C). Repeat EGD was performed 35 mo later and revealed no residual neuroendocrine tumor.

Patient 2

A 61-year-old male with a history of gastroesophageal reflux disease (GERD) underwent EGD for evaluation of chest discomfort with breakthrough reflux symptoms while taking a proton pump inhibitor daily. LA Grade C esophagitis and ulcerated mucosa were present in the distal esophagus. A 6 mm sessile polyp also observed in the duodenal bulb and resected by snare. Pathology revealed a neuroendocrine tumor of the duodenum. Positron emission tomography-computed tomography (PET-CT) was performed and demonstrated no evidence of hypermetabolic malignancy. A repeat EGD with biopsies from the previous polypectomy site six weeks later demonstrated reactive duodenopathy with foveolar metaplasia but no residual neuroendocrine tumor.

Patient 3

A 50-year-old female with chronic diarrhea was found to have metastatic liver disease of unknown primary origin on CT. The largest lesion measured 9 cm × 6 cm in the right hepatic lobe and PET-CT demonstrated only moderate metabolic activity consistent with a neuroendocrine tumor. CT-guided liver biopsy demonstrated metastatic neuroendocrine tumor with positive synaptophysin, chromogranin, NSE, and CD57 reactions on immunohistochemistry. EGD was performed that showed chronic esophagitis, hiatal hernia, and nodularity in the gastric fundus. Pathology from gastric biopsies revealed only benign lymphoid aggregates. Follow-up CT findings included a 2.4 cm partially calcified mass in the mid-abdominal mesentery suggestive of a neuroendocrine tumor of small bowel origin. The patient was started on long-acting octreotide and entered into hospice care 28 mo after initial presentation.

Patient 4

A 70-year-old male presenting with epigastric pain and 15 pound weight loss underwent upper endoscopy revealing chronic esophagitis, hiatal hernia, acute and chronic gastritis involving the antrum, and a small polypoid lesion which was found in the duodenal bulb. Biopsies were consistent with chronic duodenitis. Colonoscopy revealed one tubular adenoma < 1 cm and multiple hyperplastic polyps. A 3 cm mesenteric mass with surrounding desmoplastic reaction, small bowel thickening, and a 2 cm liver lesion were found on CT of the abdomen and pelvis (Figure 2A). CT guided biopsy of the mesenteric mass demonstrated a metastatic well-differentiated neuroendocrine tumor with immunohistochemistry positive for cytokeratin, NSE, synaptophysin, chromogranin, and CD56 (Figure 2B and C); however, biopsy of the liver lesion was negative for malignancy. PET-CT demonstrated heterogenous metabolic activity of the mesenteric mass with metabolic activity of the liver lesion similar to the surrounding hepatic parenchyma. Urinary 5-hydroxyindoleacetic acid (5-HIAA) was within normal range. The overall presentation was most consistent with a neuroen-

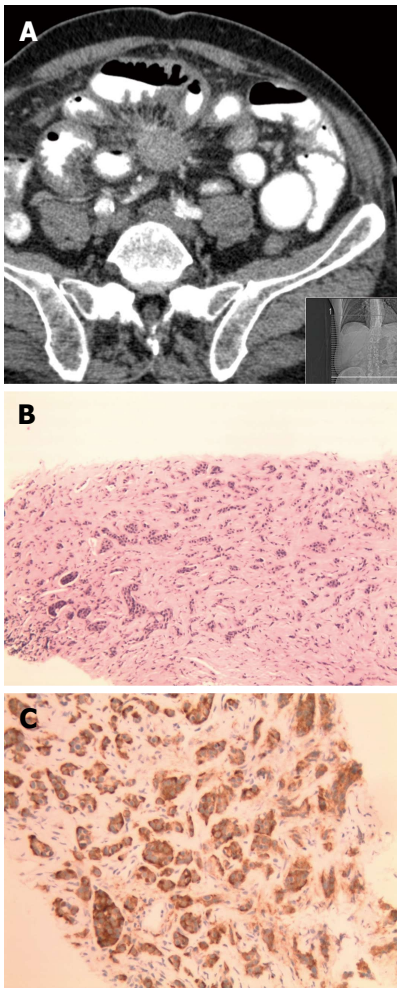

Figure 2 A 70-year-old male presenting with epigastric pain and 15 pound weight loss underwent upper endoscopy revealing chronic esophagitis, hiatal hernia, acute and chronic gastritis involving the antrum, and a small polypoid lesion which was found in the duodenal bulb. A: Patient 4, neuroendocrine (carcinoid) tumors as solid spiculated mesenteric mass on computed tomography of abdomen; B: Diffuse infiltration by monotonous bland cells with trabecular growth pattern. Mitoses, atypia and necrosis are not identified. H and E, × 10; C: The tumor cells are diffusely and strongly positive for CD56 immunohistochemical stain. CD56, × 20.

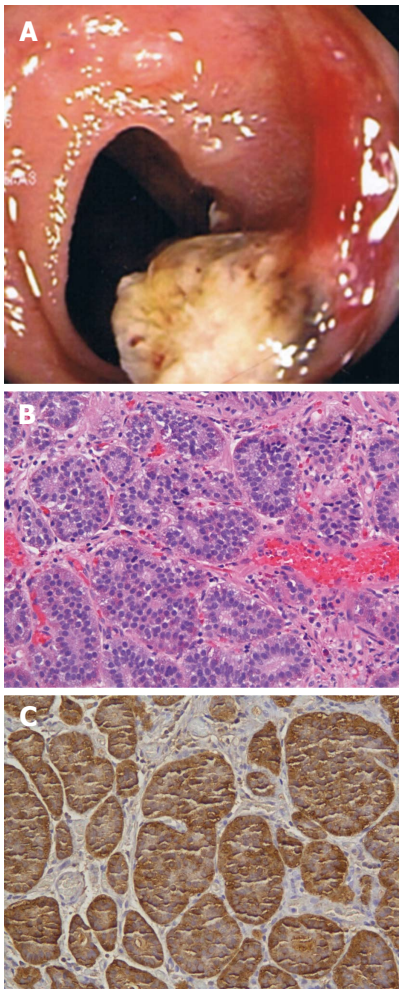

Figure 3 A 40-year-old male with recurrent perianal fistulous disease underwent colonoscopy to rule out inflammatory bowel disease. A: Patient 6, neuroendocrine (carcinoid) tumors as 10 mm ileocecal sessile polyp at colonoscopy; B: Nests of monotonous cells with bland nuclei arranged in organoid pattern. H and E, × 10; C: Carcinoid tumor; Chromogranin A: Marked cytoplasmic positivity.

docrine tumor originating in the small bowel. The patient was started on long-acting octreotide therapy and did not undergo surgical resection of the tumor.

Patient 5

A 45-year-old female presented to an outside facility with nausea, vomiting, and abdominal pain and had dilation of the small bowel and colon and ascitic fluid on CT scan. Gastrografin enema demonstrated an obstruction in the sigmoid colon. An area of desmoplastic reaction involving the sigmoid colon was found during exploratory laparotomy along with multiple metastatic lesions to the liver and mesenteric and peritoneal implants. Surgical decompression of the small bowel and colon was performed and the patient was transferred for further care. Biopsies obtained from the peritoneal implants were consistent with a low-grade neuroendocrine tumor with immunohistochemistry positive for chromogranin and synaptophysin. Serum chromogranin A level was elevated at 27 nmol/L. Following transfer to our facility, the patient underwent colonoscopy which revealed a 3 cm area of stenosis due to extrinsic compression 30 cm from the anal verge. As no further surgical intervention was deemed appropriate, two overlapping metal colonic stents (Wallstent, 22 mm × 90 mm and 22 mm × 60 mm) were placed across the area of stenosis. The patient was later discharged for hospice care.

Patient 6

A 40-year-old male with recurrent perianal fistulous disease underwent colonoscopy to rule out inflammatory bowel disease. Colonoscopy revealed normal colonic mucosa and a 1 cm sessile polyp at the terminal ileum (Figure 3A). Snare polypectomy was performed and pathology revealed a submucosal neuroendocrine tumor with well formed nests of cells and diffuse expression of synaptophysin and chromogranin. KI-67 proliferative index was < 5% (Figure 3B and C). The patient was lost to follow-up.

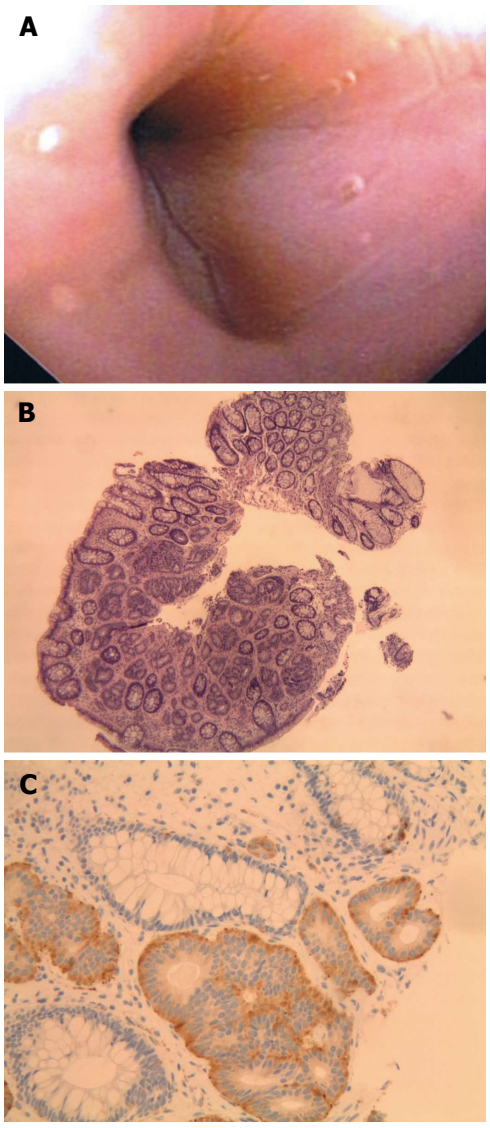

Figure 4 A 50-year-old female presented for evaluation of gastroesophageal reflux disease and colon cancer screening. A: Patient 7, neuroendocrine (carcinoid) tumors as sessile sigmoid polyp at colonoscopy; B: Organoid growth pattern with regular bland nuclei with indistinct cell borders. H and E, × 10; C: The neuroendocrine cells are positive for Synaptophysin and adjacent colonic glands are negative. Synaptophysin, × 20.

Patient 7

A 50-year-old female presented for evaluation of GERD and colon cancer screening. EGD revealed a hiatal hernia, chronic esophagitis, and chronic gastritis. On colonoscopy, benign polyps were removed from the cecum and transverse colon. A 5 mm sessile polyp resected with hot forceps in the sigmoid colon (Figure 4A); pathology demonstrated atypical proliferation of cells and glandular-like inflammation with monotonous nuclei indicative of a neuroendocrine tumor. Immunostains were positive for chromogranin, synaptophysin, and CD56 consistent with a neuroendocrine tumor (Figure 4B and C). Somatostatin receptor scintigraphy demonstrated no evidence of other carcinoid tumors. Surveillance colonoscopy performed one year later revealed a scar at the site of the previously resected tumor without tumor recurrence.

Patient 8

A 77-year-old female with Stage I right breast cancer presented for screening colonoscopy. A 7 mm submucosal nodule was biopsied from the sigmoid colon; pathology revealed tumor cells positive for synaptophysin and negative for chromogranin but overall consistent with a neuroendocrine tumor. Urinary 5-HIAA levels and octreotide scan were unremarkable. Endoscopic mucosal resection was subsequently performed with a snare. Excisional biopsy consisted of a 7 mm × 6 × mm × 3 mm submucosal neuroendocrine tumor. Colonoscopy one year later revealed no recurrence.

Patient 9

A 55-year-old male presented for evaluation of left lower quadrant tenderness and colon cancer screening. Colonoscopy revealed a 1.4 cm tubulovillous adenoma in the sigmoid colon. A 6 mm rectal polyp removed by snare was consistent with a neuroendocrine tumor. Surveillance colonoscopy three years later revealed a 6 mm hyperplastic polyp in the rectum and no evidence of recurrence of a neuroendocrine tumor.

Patient 10

A 54-year-old female presented for colon cancer screening. On colonoscopy, an 8 mm nodule was found in the rectum. Snare polypectomy was performed. Pathology demonstrated atypical proliferation of cells and glandular-like inflammation with monotonous nuclei suggestive of a neuroendocrine tumor. Colonoscopy one year later was negative for recurrence.

Patient 11

A 60-year-old female presented for colon cancer screening. On colonoscopy, a 5 mm submucosal nodule was found in the rectum and removed snare polypectomy. The biopsy was consistent with a neuroendocrine tumor involving the submucosa with tumor cells positive for synaptophysin and focally positive for chromogranin. Fourteen months later, colonoscopic biopsies from the polypectomy site revealed no recurrence.

DISCUSSION

Our case series describes a wide spectrum of benign gastrointestinal NET originating in the small intestine, colon, and rectum and malignant NET originating in the liver and intraabdominal sites. The following discussion will focus on the diagnosis and management of NET originating from the luminal gastrointestinal tract and will not include pancreatic NET.

Advances in our understanding of both the biologic and morphologic heterogeneity of NET have left the term "carcinoid" nearly obsolete[7]. Gastroenteropancreatic NET (GEP-NET), encompassing both traditional gastrointestinal carcinoids and pancreatic endocrine tumors,

Table 2 Hormone production by tumor location[15,9,31,43]

Location	Hormones
Stomach	Histamine, Gastrin, Serotonin, Somatostatin, Gastrin Releasing Peptide
Duodenum/Upper Jejunum	Gastrin, Serotonin, Somatostatin, Gastrin Releasing Peptide
Ileum/Cecum	Enteroglucagon, Serotonin, Substance P, Tachykinins
Appendix	Enteroglucagon, Peptide YY, Serotonin, Somatostatin
Colon/Rectum	Enteroglucagon, Serotonin, Somatostatin
Pancreas	ACTH, Calcitonin, Cholecystokinin, Corticotropin-Releasing Hormone, Gastrin, Glucagon, Growth Hormone-Releasing Hormone, Growth Hormone-Releasing Factor, Insulin, Neurotensin, Pancreatic Polypeptide, Parathyroid Hormone-Related Peptide, Prolactin, Somatostatin, Vasoactive Intestinal Peptide

are replacing the less descriptive and often times pathologically and clinically more confusing term "carcinoid"[3,9]. In 2000, the World Health Organization (WHO) classification replaced "carcinoid" with the terms neuroendocrine tumors and neuroendocrine carcinomas to describe gastrointestinal neoplasms originating from the diffuse system of neuroendocrine cells[9]. Along with developing tumor node metastasis staging and grading systems[10-14], the WHO classification[9] provides an improved system for determining prognosis and treatment and includes three main groups subdivided by organ of tumor origin: (1) well differentiated neuroendocrine tumors (benign behavior or uncertain malignant potential-"carcinoids"); (2) well differentiated neuroendocrine carcinomas (low-grade malignancy-"malignant carcinoids"); and (3) poorly-differentiated carcinomas (high-grade malignancy). This classification replaces the previous outdated system which was based on embryologic cell of origin (foregut, midgut, hindgut) and shared little correlation between tumor behavior and tumor location especially for neoplasms originating in the foregut (tracheobronchopulmonary, gastric, and pancreatic tumors)[3,9]. Histologically, tumor proliferation capacity is measured by Ki-67 staining with Ki-67 Index < 2% seen in grade Ⅰ tumors, 2%-20% in grade Ⅱ tumors, and > 20% tumor cell involvement in grade Ⅲ GEP-NET[11].

Cells originating from the diffuse system of neuroendocrine cells within the gastrointestinal tract share phenotypic similarities with neural cells in their expression of synaptophysin, NSE, and chromogranin A[3,10]. Useful as GEP-NET markers found on the secretory vesicles of neuroendocrine cells, these proteins usually remain independent of cellular production of hormones that are stored within the vesicles[3,10,15]. Hormone production and biologic activity generally varies by GEP-NET location (Table 2) and less than half of the known hormones originating from at least 15 different types of endocrine cells are expressed by GEP-NET[15]. Many tumors remain clinically silent and may present with intestinal obstruction as a result of tumor-induced fibrosis rather than signs or symptoms of secretory products[3]. The classic carcinoid syndrome (cutaneous flushing and secretory diarrhea) occurs in less than 10% of patients[3] and typically in the setting of hepatic metastases.

Diagnostic evaluation

Initial evaluation of patients with a suspected GEP-NET should include a serum chromogranin A level[3,16]. Elevated in approximately 80% of patients with neuroendocrine tumors regardless of location and functional activity, chromogranin A levels also appear to correlate with overall tumor burden[17]. Twenty-four-hour urinary 5-HIAA levels as well as serum gastrin, histamine, serotonin, and substance P levels should be included as part of the initial evaluation when the presentation is consistent with carcinoid syndrome[3]. Urinary 5-HIAA elevation sensitivity is as high as 100% with a specificity of 88% for the carcinoid syndrome[18]. Care must be taken to avoid medications and foods that may affect urinary 5-HIAA excretion; large amounts of serotonin are in foods as avocados, bananas, eggplant, kiwi, pineapple, plums, tomatoes, and walnuts and may cause false positive results[16].

Patients with positive biochemical markers should be evaluated with somatostatin receptor scintigraphy (^{111}Indium-labeled octreotide scan) for tumor localization as well as either CT or magnetic resonance imaging (MRI) to identify mass lesions, mesenteric fibrosis, and lymphadenopathy[3,16]. ^{111}Indium-labeled octreotide scan is useful in detection of both primary and metastatic tumors with sensitivity as high as 90%[3,19]. CT and MRI play an important role in identification of primary tumors and metastatic disease; however, they may underestimate the extent of disease in up to 25% of cases[20,21] and overall sensitivities around 80% are lower than ^{111}Indium-labeled octreotide scanning[3]. Radiolabeled metaiodobenzylguanide (^{123}I-MIBG) scanning may be used in patients on long-acting octreotide medications which interfere with somatostatin receptor scintigraphy[3]. Radiolabeled 5-HTP positron emission tomography has demonstrated better sensitivities than CT and octreotide scanning; however, it is not widely available and is generally still considered an investigational modality[3,21,22]. Barium studies, including small-bowel-follow-through, play little if any role in tumor localization with the availability of other diagnostic modalities with increased sensitivity[23].

Following tumor localization, biopsy for tissue diagnosis should be obtained including performing upper endoscopy and colonoscopy with ileoscopy as clinically indicated[3,16]. Small bowel enteroscopy has low diagnostic sensitivities as well as a limited ability to evaluate the distal jejunum and ileum and has largely been replaced by capsule endoscopy in both diagnostic and surveillance roles[3,24,25]. Endoscopic ultrasound (EUS) plays in

important role in guiding management as it is accurate in assessing tumor size and depth of invasion especially in gastric, duodenal, and rectal carcinoid tumors[26,27].

Site specific information

Gastric carcinoids are typically divided into Type Ⅰ, Ⅱ, and Ⅲ tumors with some classifications including Type Ⅳ tumors[9]. Type I and Ⅱ gastric carcinoid tumors develop in response to hypergastrinemia effects on enterochromaffin-like cells of the oxyntic mucosa found in the gastric fundus and body[28-30]. Type I are the most common gastric NET tumors usually presenting as small multifocal lesions associated with autoimmune chronic atrophic gastritis and hypergastrinemia in the setting of low gastric acid output[3,9,30]. They have an excellent prognosis with 5-year survival rates > 95%[3]. Type Ⅱ gastric NET develop in patients with Multiple Endocrine Neoplasia type-1 (MEN-1) associated Zollinger-Ellison syndrome (ZES) as a result of tumor driven hypergastrinemia in the setting of an autosomal dominant mutation of the *MEN-1* gene located on chromosome 11q13[9,29,30]. Type Ⅱ gastric NET rarely develop in patients with sporadic ZES[29]. Prognosis is good with 5-year survival rates of 70%-90%[3].

Type I and Ⅱ tumors < 1 cm in size without extension into the muscularis propria on EUS can initially be managed with endoscopic mucosal resection[3,26,31]. When more than 5 lesions are present, tumor size is > 1 cm, or recurrence occurs at a site of previous endoscopic resection, local surgical excision is recommended[3]. Type Ⅱ lesions may require aggressive gastrectomy as well as surgical resection of the underlying gastrinoma[3]. Surveillance endoscopy with biopsy should be performed every six months following both endoscopic and surgical tumor removal[3].

Type Ⅲ tumors are sporadic gastric carcinoids which develop in normal gastric mucosa in the setting of normal gastrin levels[3,9]. They are aggressive with deep invasion and the potential for metastatic disease characteristic of even small primary tumors[26]. Five-year survival rates are < 35%[3]. Type Ⅳ tumors are neuroendocrine carcinomas which are indistinguishable from gastric adenocarcinomas with the exception of the presence of neuroendocrine cells within the tumor matrix[3]. Both type Ⅲ and Ⅳ tumors should be managed surgically with complete or partial gastrectomy[3,9].

Small intestine

Duodenal: Five types of duodenal neuroendocrine tumors have been described[32]: (1) gastrinomas which may occur sporadically or in the setting of MEN-1/ZES and are the most common duodenal NET[3,9,32]; (2) somatostatinomas which usually occur in the ampullary/periampullary region and are more likely to be associated with von Recklinghausen's disease (neurofibromatosis type 1)[3,33]; (3) gangliocytic paraganglionomas[3,9,32]; (4) nonfunctioning NET which contain serotonin-, gastrin-, or calcitonin-positive cells[3,9]; and (5) neuroendocrine carcinomas[3,32,33]. Overall 5-year survival for duodenal carcinoid lesions is 60%[3]. Endoscopic resection may be considered for nonmetastatic duodenal (and ampullary) lesions measuring up to 2 cm if the tumor is confined to the mucosa and submucosa on EUS examination[3,33-35]. Surgical resection should be performed on tumors > 2 cm[34,35]. While distant metastases rarely occurs with duodenal NET, lymph node metastases may occur in tumors < 1 cm and surgical resection should be performed in all patients with evidence of lymph node involvement on pretreatment imaging studies[35].

Jejuno-Ileal: Terminal ileum NET are the most common GEP-NET. They are frequently found at an advanced stage with metastatic disease to the liver present in 50% and regional lymph node involvement in up to 70% of patients regardless of primary tumor size[21]. Associated mesenteric fibrosis, nodal metastases, and desmoplastic reactions involving mesenteric vessels may lead to nonspecific abdominal pain, gastrointestinal bleeding, intermittent ischemia, or bowel obstruction. These symptoms may prompt emergent surgical intervention and subsequent diagnosis of a previously unidentified jejunal or ileal NET in up to 40% of patients[3,21]. Ileal NET are associated with the carcinoid syndrome in the setting of liver metastases in approximately 20% of cases[9,21]. While the 5-year survival rate is 60% for both jejunal and ileal tumors, it is as low as 18% when hepatic metastases are present[3]. Surgical resection of the primary tumor as well as *en bloc* resection of regional lymph nodes is recommended and should be performed even when hepatic metastases is present in order to delay progression and local complications of disease[21,31].

Appendix: Appendiceal NET are the most common appendiceal tumor[21]. They are often found incidentally during appendectomy with the majority (90%) of tumors < 1 cm in size. Overall 5-year survival for appendiceal NETs is 98% for benign tumors and 27% for malignant tumors[3]. Metastatic disease rarely occurs with tumors < 2 cm and the occurrence of metastases increases with increasing tumor size over 2 cm[3,21,36]. Tumors > 2 cm should be managed with right hemicolectomy. Appendectomy should be performed in tumors < 2 cm in size with right hemicolectomy considered for tumors 1-2 cm based on pathologic criteria (invasion into mesoappendix, serosal or lymphovascular invasion, involvement of tumor margins, positive lymph nodes, or Ki67 index > 2% on immunohistochemistry staining)[21]. Variant mixed endocrine/exocrine goblet-cell (adenocarcinoid) tumors are more aggressive lesions associated with a poorer prognosis and higher rates of both metastatic and recurrent disease and should be managed with right hemicolectomy regardless of tumor size[21,36].

Colon: Neuroendocrine tumors rarely occur in the colon with many previously reported cecal NET representing appendiceal tumors[3,9]. Clinical presentation of colonic NET includes change in bowel habits, gastrointestinal

bleeding, abdominal pain, weight loss, and asymptomatic lesions found during screening colonoscopy is generally indistinguishable from other mass lesions of the colon[3,9]. Most tumors are > 2 cm in size with invasion into the muscularis propria at the time of diagnosis and overall prognosis is poor with 5-year survival rates of only 33%-42%[3]. Wide surgical resection with lymph node dissection is recommended for management of colonic NET[3] as metastatic disease is common at the time of diagnosis[9]. Local excision may be considered for tumors < 2 cm in size[3]; however, data regarding metastatic disease in this setting are limited.

Rectum: Frequently found as small, asymptomatic submucosal tumors during endoscopic evaluation, rectal NET have an excellent overall prognosis with 5-year survival rates of 87%[3,9]. When present, symptoms may include change in bowel habits, gastrointestinal bleeding, anorectal discomfort, and pruritis ani[3]. Submucosal tumors < 1 cm in size account for 80% of rectal carcinoids[3] and can be managed endoscopically in the absence of muscularis invasion or pararectal lymph node metastases on EUS examination[31,37]. Rectal NETs 1-2 cm in size may be managed with wide surgical excision if there is no evidence of muscularis invasion or lymph node metastasis[3]. Low anterior resection or abdominoperineal resection is recommended for tumors > 2 cm as the risk of metastatic disease increases with tumors > 2 cm in size and with invasion of the muscularis propria[3,9].

Medical therapy

Following surgical resection of a GEP-NET, medical therapy may be required for symptom management related to functional tumor syndromes as well as management of progressive metastatic and residual disease[31,38,39]. Patients with symptomatic functional NET should be considered for somatostatin (SST) analog (short- or long-acting octreotide) or interferon- α therapy alone or in combination[3,31,38,39]. In addition to reducing symptoms in patients with carcinoid syndrome[40,41], VIPoma associated Verner-Morrison syndrome (watery diarrhea, hypokalemia, and achlorhydria)[40,42], and glucagonoma associated necrolytic migratory erythema[4], SST analogs may also play a role in growth inhibition of nonfunctioning NET[39,41]. Interferon-α therapy may be considered in patients who become intolerant or resistant to SST analog therapy as it has also been shown to reduce diarrhea and flushing in patients with carcinoid syndrome[39].

Systemic chemotherapy or peptide receptor radionuclide therapy with I-131 MIBG, Yttrium^{90}, or Lutetium^{177} should be considered in patients with metastatic disease with transarterial embolization/chemoembolization or radiofrequency ablation considered when metastases are limited to the liver[3,31,38,39].

Patients undergoing biologic or cytotoxic therapies should have their clinical response to treatment monitored every 3 mo[11]. Biochemical markers (based on the functional status of their underlying tumor) should be followed every 3-6 mo along with CT or MRI scanning every 6 mo for 5 years following curative surgical resection[11].

In a conclusion, GEP-NET are relatively rare neoplasms of the gastrointestinal tract with variable clinical presentation, morbidity, and mortality dependent on tumor location, metastatic potential, and functional biologic status. Staging and classification systems for GEP-NET are likely to continue to evolve along with further development of tumor directed diagnostic and therapeutic modalities as our understanding of GEP-NET continues to expand over time.

COMMENTS

Case characteristics

This case series describes a wide spectrum of benign gastrointestinal neuroendocrine (carcinoid) tumors (NET).

Clinical diagnosis

The diagnosis and management of NET originating from the luminal gastrointestinal tract and will not include pancreatic NET.

Imaging diagnosis

Computed tomography and magnetic resonance imaging play an important role in identification of primary tumors and metastatic disease.

Experiences and lessons

Gastroenteropancreatic NET are relatively rare neoplasms of the gastrointestinal tract with variable clinical presentation, morbidity, and mortality dependent on tumor location, metastatic potential, and functional biologic status.

Peer review

This is a very good example of a case series combined with a good review.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flex-

ible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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