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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: wjgo@wjgnet.com
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FIELD OF VISION

Epigenetic field defects in progression to cancer

Carol Bernstein, Valentine Nfonsam, Anil Ramarao Prasad, Harris Bernstein

Carol Bernstein, Harris Bernstein, Department of Cellular and Molecular Medicine, College of Medicine, University of Arizona, Tucson, AZ 85724, United States
Valentine Nfonsam, Department of Surgery, College of Medicine, University of Arizona, Tucson, AZ 85724, United States
Anil Ramarao Prasad, Department of Pathology, College of Medicine, University of Arizona, Tucson, AZ 85724, United States
Author contributions: Bernstein C wrote the manuscript; Nfonsam V and Prasad AR collected materials and discussed the topic; Bernstein H revised the manuscript.
Correspondence to: Carol Bernstein, PhD, Department of Cellular and Molecular Medicine, College of Medicine, University of Arizona, Tucson, AZ 85724,
United States. bernstein324@yahoo.com
Telephone: +1-520-2415260 Fax: +1-520-7950073
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Abstract

A field defect is a field of pre-malignant tissue in which a new cancer is likely to arise. Field defects often appear to be histologically normal under the microscope. Recent research indicates that cells within a field defect characteristically have an increased frequency of epigenetic alterations and these may be fundamentally important as underlying factors in progression to cancer. However, understanding of epigenetic field defects is at an early stage, and the work of Katsurano *et al* published this year, is a key contribution to this field. One question examined by Katsurano *et al* was how early could the formation of an epigenetic field defect be detected in a mouse colitis model of tumorigenesis. They highlighted a number of measurable epigenetic alterations, detected very early in normal appearing tissue undergoing histologically invisible tumorigenesis. They also documented the increasing presence of the epigenetic alterations at successive times during progression to cancer. In this commentary, we offer a perspective on the changes they observed within a broader sequence of epigenetic events that occur in progression to cancer. In particular, we highlight the likely central role of epigenetic deficiencies in DNA repair gene expression that arise during progression to cancer.

Key words: Field defect; Epigenetics; Tumorigenesis; Carcinogenesis; DNA damage; DNA repair; Colon cancer; Mouse; Human

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COMMENTARY ON HOT TOPICS

We read with great interest the recent article by Katsurano *et al*[1] describing a mouse colitis model leading to tumor formation. They report that an epigenetic field defect forms early after treatment with dextran sulfate sodium (DSS) and that the epigenetic alterations continue to increase even with diminishing stimulation. Their study was unique in showing that particular epigenetic alterations, involving DNA methylation, increased even while inflammation was diminishing.

The term "field cancerization" was first used in 1953 to describe an area or "field" of epithelium that has been preconditioned by (at that time) largely unknown processes so as to predispose it towards development of cancer[2]. Since then, the terms "field cancerization" and "field defect" have been used to describe pre-malignant tissue in which new cancers are likely to arise.

Field defects are of crucial importance in progression to cancer, though they have not received a great deal of attention thus far. As pointed out by Rubin[3], "The vast majority of studies in cancer research has been done on well-defined tumors *in vivo*, or on discrete neoplastic foci *in vitro*. Yet there is evidence that more than 80% of the somatic mutations found in mutator phenotype human

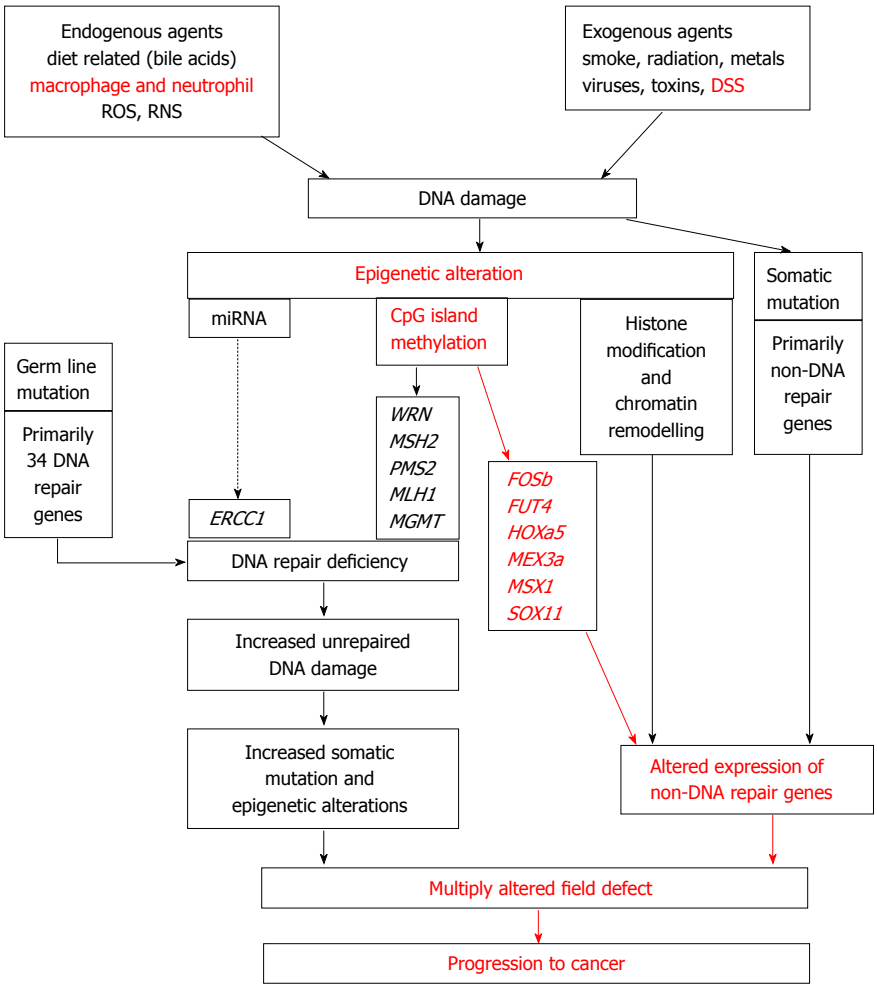

Figure 1 Roles of DNA damage, reduced DNA repair and epigenetic alterations in a field defect in progression to cancer.

colorectal tumors occur before the onset of terminal clonal expansion[4].” Field defects with mutations are precursors of cancers with those same mutations. Likewise, epigenetic alterations in field defects are precursors of cancers with those same epigenetic alterations.

Colon cancers contain a median of 76 non-silent sequence mutations, of which about 15 are “driver mutations” (the rest are “passenger mutations”)[5], as well as about 55 aneuploidy events[6]. By comparison, some frequent epigenetic alterations in colon cancers affect hundreds of genes. For example, CpG island methylation of the DNA sequence encoding microRNA miR-137 reduces its expression, and this is a frequent early epigenetic event in colorectal carcinogenesis, occurring in 81% of colon cancers and in 14% of the normal appearing colonic mucosa of the field defects associated with these cancers[7]. Silencing of miR-137 can affect expression of 491 genes, the targets of this miRNA[7]. Changes in the level of miR-137 expression result in changed mRNA expression of the target genes by 2 to 20-fold and corresponding, though often smaller, changes in expression of the protein products of the genes. Other microRNAs, with likely comparable numbers of target genes, are even more frequently epigenetically altered in colonic field defects and in the colon cancers that arise from them. These include miR-124a, miR-34b/c and miR-342 which are silenced by CpG island methylation of their encoding DNA sequences in primary tumors at rates of 99%, 93% and 86%, respectively, and in the adjacent normal appearing mucosa at rates of 59%, 26% and 56%, respectively[8,9]. In addition to epigenetic alteration of expression of miRNAs, other common types of epigenetic alterations in cancers that change gene expression levels include direct hypermethylation or hypomethlyation of CpG islands of protein-encoding genes and alterations in histones and chromosomal architecture that influence gene expression[10,11].

The specific epigenetic alterations studied by Katsurano *et al*[1], as well as epigenetic field defects in general, can be placed in a broad explanatory framework starting with the occurrence of DNA damage, a major primary event in progression to cancer as shown in Figure 1. In Figure 1 italicized capitalized abbreviations are symbols of epigenetically altered genes. *ERCC1, WRN, MSH2, PMS2, MLH1* and *MGMT* are symbols for specific DNA repair genes. *FOSb, FUT4, HOXa5, MEX3a, MSX1, SOX11* are symbols for epigenetically altered genes described by Katsurano *et al*[1]. These symbols for the genes (in the

order listed above) are: *FBJ* osteosarcoma oncogene B, fucosyltransferase 4, homeobox A5, mex3 homolog A, homeobox msh-like 1, and SRY-box-containing gene 1, respectively.

DNA damage as a primary cause of cancer

Exogenous and endogenous agents that induce DNA damage have been identified as major causes of many common cancers (Figure 1). These include cancers of the lung (tobacco smoke[12]), colorectum (exposure to bile acids that cause increased reactive oxygen species (ROS) and reactive nitrogen species, and are produced in response to a high fat diet[13]), esophagus (exposure to stomach acids plus bile acids due to gastroesophageal reflux[14]), stomach (reactive oxygen species caused by *Helicobacter pylori* infection[15]), liver (*Aspergillus* metabolite aflatoxin B_1[16]), cervix/uterus (human papillomavirus plus increased nitric oxide from tobacco smoke or other infection[17]) and melanoma (UV light from solar radiation[18]). Inherited germ line mutations in DNA repair genes similarly cause an increase in DNA damages due to a deficiency in repair capability, and these also cause increases in cancer risk. At least 34 inherited human DNA repair gene mutations increase cancer risk, including, for example, germ line mutations in the *BRCA1, XPC* and *MLH1* genes[19]. From a study of 44 788 pairs of twins, it is estimated that overall, about 30% of cancers are familial (largely due to inherited germ line mutations or genetic polymorphisms) and 70% are sporadic[20].

DNA damages cause epigenetic changes and mutations

ROS, produced during inflammation and other types of cellular stress, cause a variety of types of DNA damage[21], some of which lead to double strand breaks[22]. During repair of double strand breaks and other types of oxidative DNA damages, methylation of promoter CpG islands in DNA and/or modification of histones can occur, causing gene silencing (Figure 1)[23,24]. These epigenetic alterations are sometimes not reversed after repair is completed[23,24]. While it has long been known that oxidative damage can cause mutation[21], it has only recently become clear that oxidative damage can also give rise to epigenetic changes (epimutation)[23,24].

Other types of DNA damage can also give rise to epimutation during DNA repair. The DNA repair enzyme Parp1 [poly(ADP)-ribose polymerase-1] acts at sites of DNA damage, especially single strand breaks, where it adds poly(ADP)-ribose to specific proteins as part of the overall DNA repair process[25]. This, in turn, directs recruitment and activation of the chromatin remodeling protein ALC1 to cause nucleosome remodeling[26]. Nucleosome remodeling has been found to cause, for instance, epigenetic silencing of DNA repair gene *MLH1*[27]. In addition, certain chemicals previously identified as DNA damaging agents, including benzene, hydroquinone, styrene, carbon tetrachloride and trichloroethylene, cause considerable hypomethylation of DNA, leading to epigenetic modifications, and some of this hypomethylation occurs through the activation of oxidative stress pathways[28].

Epigenetic changes in DNA repair gene expression are a likely source of genomic instability

While germ line (familial) mutations in DNA repair genes cause a high risk of cancer, in sporadic (non-familial) cancers, by contrast, somatic mutations in DNA repair genes are rarely found[5]. However, deficient expression of DNA repair genes is frequently observed within sporadic cancers, and this is almost always due to epigenetic alteration (Figure 1). Epimutation leading to silencing of a gene necessary for DNA repair will allow unrepaired damages to increase. Such additional DNA damages, in turn, will cause increased mutations and epimutations, including carcinogenic driver mutations and epimutations.

Truninger *et al*[29] compared the frequencies of germ line mutations, CpG island methylations and other unidentified alterations in the down-regulation of expression of DNA mismatch repair (MMR) gene *MLH1* in colon cancer. They evaluated 1 048 unselected consecutive colon cancers. They found that 103 of these cancers were deficient in protein expression of *MLH1*. Among the MLH1 deficient cancers, 68 were sporadic and the remaining 35 were due to germ line mutations. Among the 68 sporadic MLH1 protein-deficient colon cancers, 65 (96%) were deficient due to epigenetic methylation of the CpG island of the *MLH1* gene. Reduced protein expression of *MLH1* in the remaining 3 sporadic MLH1 protein-deficient cancers may have been caused by over expression of the microRNA miR-155. This explanation is suggested by the finding that transfection of miR-155 into cells caused reduced expression of *MLH1*[30]. Furthermore, high expression of miR-155 was found in colon cancers in which protein expression of *MLH1* was reduced and the *MLH1* gene was neither mutated nor hypermethylated[30].

Some of the epigenetic alterations in DNA repair genes found in colon cancers, as well as in their associated field defects, are summarized in Table 1[31-35].

Deficiencies in DNA repair genes cause increased mutation rates in MMR defective cells[36,37] and in homologous recombinational repair (HRR) defective cells[38]. Chromosomal rearrangements and aneuploidy also increase in HRR defective cells[39]. Thus, deficiency in DNA repair causes genomic instability (a mutator phenotype), the likely main underlying cause of DNA sequence alterations leading to tumorigenesis. Genomic instability permits the acquisition of a sufficient number of mutations and epimutations in tumor suppressor genes and oncogenes to fuel carcinogenesis. Deficiencies in DNA repair appear to be central to the genomic and epigenomic instability characteristic of cancer.

Figure 1 illustrates the chain of consequences of exposure of cells to endogenous and exogenous DNA damaging agents that lead to cancer. The role of germ line defects in DNA repair genes in familial cancers are also indicated. The large role of DNA damage and con-

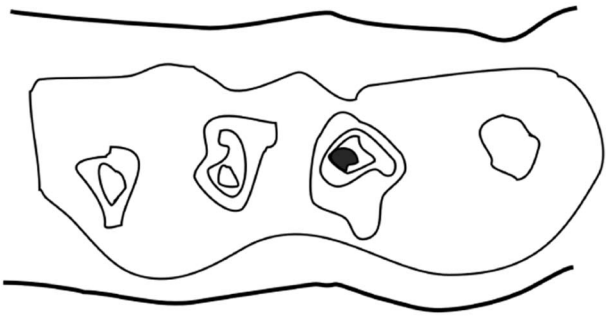

Figure 2 Schematic of a field defect in progression to cancer.

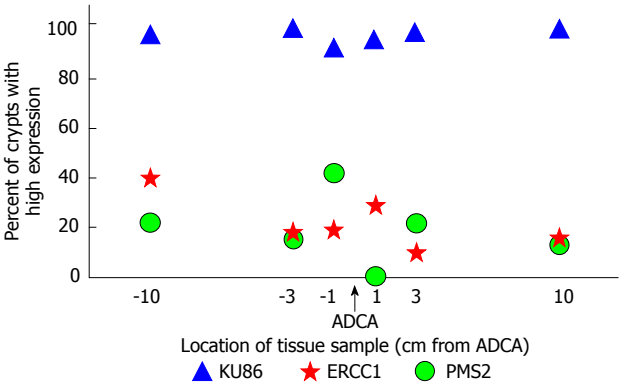

Figure 4 Reduced expression of DNA repair proteins ERCC1 and PMS2 and lack of reduction of DNA repair protein KU86 at distances up to 10 cm from an adenocarcinoma in a colon resection (graphed here from data reported on one of 8 similarly affected colon resections). ADCA: Adenocarcinoma.

sequent epigenetic DNA repair deficiencies leading to sporadic cancer are emphasized. The role of directly induced somatic mutation in sporadic cancer is indicated as well. The items shown in red lettering were demonstrated in the recent article of Katsurano *et al*[1].

Sequence of epimutation, mutation and natural selection leading to carcinogenesis

A field defect arises when an epimutation or mutation occurs in a stem cell that provides a reproductive advantage allowing clonal descendents of that stem cell to outcompete neighboring stem cells. These cells form a patch of somewhat more rapidly growing cells (an initial field defect). As the patch enlarges at the expense of neighboring cells, an additional epimutation or mutation may arise in one of the field defect stem cells so that this new stem cell with two advantageous epimutations and/or mutations generates daughter stem cells that can out-compete the surrounding field defect of cells that have just one advantageous epimutation or mutation. As illustrated in Figure 2, this process of expanding sub-patches within earlier patches can occur multiple times until a particular constellation of epimutations and mutations results in a cancer (represented by the small dark patch in Figure 2). The cancer, once formed, continues to evolve and to produce sub clones. A renal cancer, for example, sampled in 9 areas, had 40 ubiquitous mutations, 59 mutations

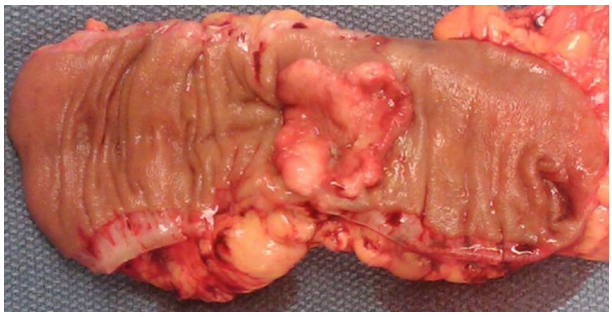

Figure 3 Human colon resection about 17 cm long, opened longitudinally to show inner epithelium, with cancer in center of inner surface.

shared by some, but not all regions, and 29 "private" mutations only present in one region[40].

Figure 3 shows an opened resected segment of a human colon that has a colon cancer. There are about 100 colonic microscopic epithelial crypts *per* sq mm in the human colonic epithelium[41]. The colonic epithelium in the resection shown in Figure 3 has an area of about 6.5 cm by 17 cm, or 111 sq cm, or 11 100 sq mm. Thus this area has about 1.11 million crypts. There are 10-20 stem cells at the base of each colonic crypt[42,43]. Therefore there are likely 11 to 22 million stem cells in the grossly unremarkable colonic mucosal epithelium shown in Figure 3. Evidence reported by Facista *et al*[35], listed in Table 1 and illustrated in Figure 4, indicates that in many such resections, most of the crypts, and thus the stem cells in such an area up to 10 cm distant (in each direction) from a colon cancer (such as in the grossly unremarkable area shown in Figure 3), and the majority of their differentiated daughter cells, are epigenetically deficient for protein expression of the DNA repair genes *ERCC1* and *PMS2*, although the epithelium is histologically normal.

The stem cells most distant from the cancer as well as those closer to the cancer in the resection defined by the data in Figure 4 appear to be deficient throughout the field defect for *ERCC1* and *PMS2*. The field defect of Figure 4, containing tens of millions of stem cells, presumably arose from an initial progenitor stem cell deficient in DNA repair (due to epigenetic silencing). Because of this repair deficit, the initial stem cell was genetically unstable, giving rise to an increased frequency of epimutations and mutations in its descendents. One daughter stem cell among its descendents presumably had a mutation or epimutation that, by chance, provided a replicative advantage. This descendent then underwent clonal expansion by natural selection because of its replicative advantage. Among the further descendents of the clone, new mutations and epimutations arose frequently, since these descendents had a mutator phenotype[44], due to the repair deficiency passed down epigenetically from the original repair-defective stem cell. Among these new mutations and epimutations, some would provide further replicative advantages, giving rise to a succession of more aggressively growing sub clones (inner rings in Figure 2), and eventually to a cancer.

Table 1 Examples of epigenetic alterations (epimutations) of DNA repair genes in colon cancers and in their field defects, with CpG island methylation indicated where known

Reference	Epimutations in genes found in colon cancer (mechanism)	Percentage of the sporadic cancers with that epimutation	Epimutations in genes in field defect (mechanism)	Percentage of the field defect with that epimutation
Agrelo *et al*[31]	*WRN* (CGI)	38%		
Shen *et al*[32]	*MGMT* (CGI)	46%	*MGMT* (CGI)	23%
Psofaki *et al*[33]	*MGMT* (CGI)	90%		
Psofaki *et al*[33]	*MLH1* (CGI)	65%		
Truninger *et al*[29]	*MLH1* (CGI)	96%		
Lee *et al*[34]	*MLH1* (CGI)	2%		
Lee *et al*[34]	*MSH2* (CGI)	13%	*MSH2* (CGI)	5%
Lee *et al*[34]	*MGMT* (CGI)	47%	*MGMT* (CGI)	11%
Facista *et al*[35]	*ERCC1*	100%	*ERCC1*	60%
Facista *et al*[35]	*PMS2*	88%	*PMS2*	50%
Facista *et al*[35]	*XPF*	55%	*XPF*	40%

CGI: CpG island methylation.

The study by Katsurano *et al*[1] identified 14 genes that were epigenetically silenced or considerably reduced in expression due to CpG island methylation within at least 4 out of 5 of the cancers arising in their DSS induced mouse model of colon cancer. These appear to be "driver" epimutations. They then evaluated the non-neoplastic epithelial cells in the scraped off distal half of mouse colons undergoing DSS-induced tumorigenesis at 2 wk, 5 wk, 8 wk and 15 wk after transitory initial exposure of the mice to DSS. These epithelial cells constitute a field defect from which a mouse colon cancer is likely to arise, since 80%-100% of the mouse colons in their repeated experiments developed tumors 15 wk after exposure to DSS. By 5 to 8 wk after DSS exposure, and before any grossly visible tumors had formed, 6 of the possible "driver" epimutations present in the cancers were not only present, but were also increasing in extent with time, in the mouse colonic field defect.

Based on their own experiments and the literature, Katsurano *et al*[1] proposed that macrophages and neutrophils in the mouse colonic epithelium were the source of reactive oxygen species causing the DNA damage that initiated the tumorigenesis (Figure 1). However, even though these inflammatory cells were diminishing in frequency in the epithelium by 2 wk after their initial great increase upon DSS exposure, the level of CpG island methylation of the 6 possible "driver" genes *FOSb, FUT4, HOXa5, MEX3a, MSX1* and *SOX11* continued to increase in the isolated epithelial cells. This increase in the percentage of CpG island methylation of these 6 genes, as tumorigenesis progressed, may have been due to clonal expansion of epithelial cells that initially had these 6 methylated genes.

The work by Katsurano *et al*[1] constitutes the first mouse model of carcinogenesis in which the unique finding was made that DNA methylation frequency of some genes increased even as the initial inflammation causing DNA damage was decreasing. This work adds important experimental support for the idea that epimutation, natural selection and clonal expansion are key factors driving colon carcinogenesis.

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ORIGINAL ARTICLE

CagA EPIYA polymorphisms in Colombian *Helicobacter pylori* strains and their influence on disease-associated cellular responses

Carlos Alberto Fajardo, Andrés Javier Quiroga, Andrea Coronado, Karen Labrador, Nicole Acosta, Pilar Delgado, Carlos Jaramillo, María Mercedes Bravo

Carlos Alberto Fajardo, Andrés Javier Quiroga, Andrea Coronado, Karen Labrador, María Mercedes Bravo, Cancer and Infectious Agents Research Group, National Cancer Institute, Bogotá 110411, Colombia
Nicole Acosta, Pilar Delgado, Carlos Jaramillo, Molecular Diagnostics and Bioinformatics Laboratory, Biological Sciences Department, Los Andes University, Bogotá 111711, Colombia
Author contributions: Bravo MM and Quiroga AJ designed the study; Fajardo CA, Quiroga AJ, Coronado A, Labrador K and Acosta N performed the research; Fajardo CA, Quiroga AJ, Coronado A, Labrador K, Acosta N, Delgado P, Jaramillo C and Bravo MM analyzed the data; Fajardo CA and Bravo MM wrote the paper.
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Correspondence to: María Mercedes Bravo, MS, Head, Cancer and Infectious Agents Research Group, National Cancer Institute, Bogotá 110411, Colombia. mbravo@cancer.gov.co
Telephone: +57-1-3341111 Fax: +57-1-3341360
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Abstract

AIM: To investigate the influence of the CagA diversity in *Helicobacter pylori* (*H. pylori*) strains from Colombia on the host cell biology.

METHODS: Eighty-four *H. pylori-cagA* positive strains with different Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs patterns, isolated from patients with gastritis (n = 17), atrophic gastritis (n = 17), duodenal ulcer (n = 16), intestinal metaplasia (n = 16) and gastric cancer (n = 18), were included. To determine the integrity of the cag pathogenicity island (*cag*PAI) we evaluated the presence of *cagA*, *cagT*, *cagE*, and *cag10* genes by polymerase chain reaction. AGS gastric epithelial cells were infected with each strain and assayed for translocation and tyrosine phosphorylation of CagA by western blot, secretion of interleukin-8 (IL-8) by enzyme-linked immuno sorbent assay after taking supernatants from cocultures and cell elongation induction. For cell elongation quantification, coculture photographs were taken and the proportion of "hummingbird" cells (> 15 μm) was determined.

RESULTS: Overall 72% (60/84) of the strains were found to harbor a functional *cag*PAI. Levels of phosphorylated CagA were significantly higher for isolates from duodenal ulcer than the ones in strains from gastritis, atrophic gastritis, intestinal metaplasia and gastric cancer (49.1% ± 23.1% *vs* 21.1% ± 19.5%, $P < 0.02$; 49.1% ± 23.1% *vs* 26.2% ± 14.8%, $P < 0.045$; 49.1% ± 23.1% *vs* 21.5% ± 19.5%, $P < 0.043$ and 49.1% ± 23.1% *vs* 29.5% ± 27.1%, $P < 0.047$ respectively). We observed variable IL-8 expression levels ranging from 0 to 810 pg/mL and from 8.8 to 1442 pg/mL at 6 h and 30 h post-infection, respectively. *cag*PAI-defective strains did not induce detectable levels of IL-8 at 6 h post-infection. At 30 h post-infection all strains induced IL-8 expression in AGS cells, although *cag*PAI-defective strains induced significantly lower levels of IL-8 than strains with a functional *cag*PAI (57.1 ± 56.6 pg/mL *vs* 513.6 ± 338.6 pg/mL, $P < 0.0001$). We did not observe differences in the extent of cell elongation induction between strains with a functional or a defective *cag*PAI in 6 h cocultures. At 24 h post infection strains with functional *cag*PAI showed high diversity in the extent of hummingbird phenotype induction ranging from 7% to 34%. *cag*PAI defective strains induced significantly lower levels of elongation than strains with functional *cag*-PAI with one or more than one EPIYA-C motif (15.1% ± 5.2% *vs* 18.9% ± 4.7%, $P < 0.03$; and 15.1% ± 5.2% *vs* 20.0% ± 5.1%, $P < 0.003$ respectively). No differences were observed in cellular elongation induction

or IL-8 expression among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 24 h of coculture. There were no associations between the levels of induction of cell elongation or IL-8 expression and number of EPIYA motifs or pathology.

CONCLUSION: The present work describes a lack of association between *H. pylori* CagA protein EPIYA motifs variations from Colombian isolates and disease-associated cellular responses.

Key words: *Helicobacter pylori*; *cagA* 3′ region; CagA protein; Interleukin 8; Cell elongation; Glu-Pro-Ile-Tyr-Ala

Fajardo CA, Quiroga AJ, Coronado A, Labrador K, Acosta N, Delgado P, Jaramillo C, Bravo MM. CagA EPIYA polymorphisms in colombian *Helicobacter pylori* strains and their influence on disease-associated cellular responses. *World J Gastrointest Oncol* 2013; 5(3): 50-59 Available from: URL: http://www.wjgnet.com/1948-5204/full/v5/i3/50.htm DOI: http://dx.doi.org/10.4251/wjgo.v5.i3.50

INTRODUCTION

Helicobacter pylori (*H. pylori*) infects more than 50% of the world's population[1]. This pathogen has been associated with the development of chronic gastritis, duodenal ulcers and gastric cancer[2], and was classified as a type I carcinogen by the International Agency for Research on Cancer[3].

One of the most important virulence factors of *H. pylori* is the *cag* pathogenicity island (*cag*PAI), which encodes for a type Ⅳ secretion system (T4SS)[4]. Also encoded in the *cag*PAI is the CagA protein, which is translocated into gastric epithelial cells through the T4SS[5], where it undergoes phosphorylation by members of the SRC and Abl families of kinases on tyrosine residues within the C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs[6-8]. Phosphorylated CagA interacts with the cellular phosphatase SHP-2[9], which in turn activates several signaling pathways involved, among others, in actin cytoskeletal rearrangements, leading to cell elongation (also known as the "hummingbird phenotype")[10,11]. Translocated CagA can also induce a proinflammatory response, resulting in the expression of interleukin-8 (IL-8) through the activation of nuclear factor κ B (NF-κB)[12-14].

CagA varies in size, and this variation has been shown to be due to EPIYA motifs repeats within the C-terminal region of the protein[15,16]. Four types of EPIYA motifs have been described (A, B, C and D) based on the sequence flanking the motif[17]. Western *H. pylori* isolates have shown to harbor combinations of type A, B and C motifs, while East Asia isolates harbor combinations of type A, B and D motifs[17,18].

A positive association between the number of EPIYA motifs repeats and the phosphorylation of CagA protein has been reported[17,19]. Several studies have shown that strains with higher numbers of EPIYA-C motifs are more closely associated with gastric cancer[20-22].

IL-8 expression in gastric tissue has been reported to correlate with the histopathological severity in *H. pylori*-positive patients[23,24]. Furthermore, it has been shown that strains with higher number of EPIYA-C motifs significantly increased IL-8 expression in gastric epithelial cells[25]. As with IL-8 expression, CagA proteins with higher number of EPIYA motifs, and especially EPIYA-C motifs, have shown to potentiate cell elongation in AGS cells[17,19,26,27].

The aim of this study was to evaluate the possible association between CagA EPIYA motifs variations in *H. pylori* isolates from Colombia with the phosphorylation of CagA protein, the expression of IL-8 and cell elongation induction in gastric epithelial cells. Associations between disease severity and *H. pylori*-induced cellular responses *in vitro* were also evaluated.

MATERIALS AND METHODS

H. pylori strains

In total, 84 *cagA*-positive and 6 *cag*PAI-negative strains obtained from the stock collection at the Instituto Nacional de Cancerología, in Bogotá, Colombia were included in the study. *CagA*-positive strains were isolated from patients diagnosed with gastritis (n = 17), atrophic gastritis (n = 17), duodenal ulcer (n = 16), intestinal metaplasia (n = 16) and gastric cancer (n = 18). Isolates' *cagA* genotyping was reported previously[28] and EPIYA motifs combinations used in this study are summarized in Figure 1. *cagA*-positive reference strain NCTC 11 637, with an ABCCC polymorphism, was used as a positive control.

Detection of cagPAI genes

H. pylori genomic DNA was obtained from plate cultures of each isolate using DNAzol (Invitrogen) extraction method according to the manufacturer's instructions. The primers used in this study are listed in Table 1. To determine the integrity of *cag*PAI we evaluated the presence of *cagA, cagT, cagE* and *cag10* genes. All PCR reactions were performed in a volume of 25 μL containing 10 mmol/L Tris, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 25 pmol of the primers, 100 ng of *H. pylori* genomic DNA and 1U Taq polymerase. The polymerase chain reaction (PCR) conditions for each reaction were previously described[29,30]. Positive (strain 11637) and negative controls (strain 3062) for the *cag*PAI were included in each run. PCR products were analyzed by agarose gel electrophoresis with ethidium bromide staining.

Culture of H. pylori strains

H. pylori strains were grown on blood agar plates, supplemented with 7% horse serum (Invitrogen), 1% Vitox (Oxoid), and Campylobacter selective supplement (Oxoid), at 37 ℃ in a 10% CO_2-humidified atmosphere for

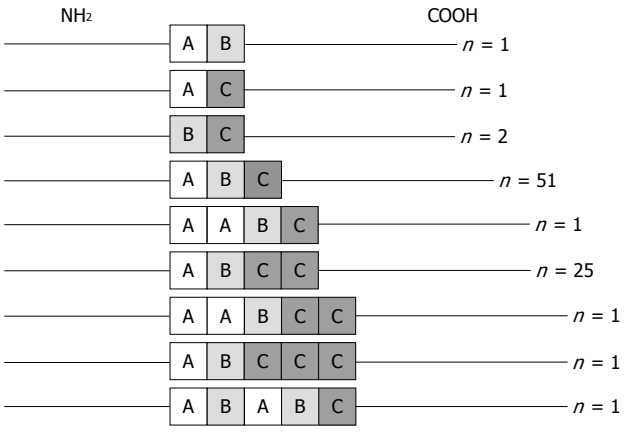

Figure 1 ***Helicobacter pylori*** **CagA Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs variations included in this study.** Eighty-four *cagA*-positive strains isolated from Colombian patients were evaluated. All strains possessed Western EPIYA motifs (A, B and C)[17] ranging from 2 to 5 in number.

3 d. Grown plates were subcultured into brucella broth (DIFCO) containing 10% horse serum (Invitrogen) and Campylobacter selective supplement (Oxoid), and were incubated under microaerophilic and shaking conditions for 24 h. Overnight cultures were set to an optical density of 0.1 at 600 nm (approximately 1.2×10^8 bacteria/mL) by dilution. Brucella broth was discarded after centrifugation of liquid cultures at 7000 rpm for 10 min and bacteria were resuspended in serum- and antibiotic-free RPMI medium (GIBCO) prior to infection.

Co-culture assays

AGS epithelial cells were seeded into 6-well plates (4×10^5 cells/well) or 25 cm^2 flasks (5×10^5 cells) and grown in RPMI 1640 (GIBCO) supplemented with 10% fetal bovine serum (GIBCO), 100 U/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen) and 2.5 μg/mL amphotericin (GIBCO) at 37 ℃ in a 5% CO_2 atmosphere for 24 h. Eighty percent confluent cell cultures were then washed with phosphate buffered saline (PBS), and serum- and antibiotic-free RPMI was added to the wells. Sixteen hours serum-starved cell cultures were infected with *H. pylori* suspensions at a multiplicity of infection (MOI) of 100. Cocultures were incubated at 37 ℃ in a 5% CO_2-humidified atmosphere.

CagA phosphorylation assays

After 6 h of coculture the medium was removed and cells were washed with PBS containing 1.0 mmol/L $CaCl_2$ and 0.5 mmol/L $MgCl_2$ and scraped from the flasks into 3 mL PBS containing 1 mmol/L sodium vanadate, harvested by centrifugation at 1000 *g* by 10 min, resuspended in 100 μL of PBS-sodium vanadate and lysed with 4 × Laemli sample buffer [0.2 TRIS-HCl pH 6.8, 0.4 mmol/L ditiothreitol, 8% sodium dodecyl sulfate (SDS), 40% glycerol, 0.4% bromophenol blue]. Cell lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a 6% resolving gel and a 4% stacking gel. Proteins were then transferred onto nitrocellulose membranes by semidry transfer. For protein detection, membranes were probed with a 1:1000 dilution of anti-phospho-tyrosine monoclonal antibody (Santa Cruz Biotechnologies) followed by a 1:4000 dilution of HRP-conjugated goat anti-mouse (Zymax, Invitrogen). Blots were developed using the Amersham ECL detection reagents (GE Healthcare). Membranes were subsequently stripped (using a 62.5 mmol/L TRIS-HCL pH 6.8, 100 μmol/L ß-2-Mercaptoethanol solution, 2% SDS at 50 ℃ for 30 min) and reprobed with 1:1000 polyclonal anti-CagA antibody (Santa Cruz Biotechnologies) followed by 1:60 000 HRP-conjugated goat anti-rabbit secondary antibody (Zymax, Invitrogen) and developed as described above. Densitometry was performed using a Gel Doc GS-670 (Biorad) and results were expressed as the ratio of phosphorylated CagA to total CagA multiplied by 100.

IL-8 assay

Medium samples from 6 h and 30 h co-cultures were collected, centrifuged at 7000 rpm for 10 min to discard unattached bacteria or cells, and supernatants were stored at -80 ℃ until further use. IL-8 concentration was measured using an IL-8 Human ELISA kit (Invitrogen) according to the manufacturer's instructions. Uninfected AGS cells were used as a negative control.

Cellular elongation assay

Six hours and twenty-four h cocultures were examined by differential interference contrast microscopy with a Leica DM IL phase contrast inverted microscope (Leica). For this, 3 randomly chosen 20 × fields were photographed with a MD800-CK camera for microscope (Amscope). Hummingbird cells were measured and counted with the software ImageJ v1.44c (developed by Wayne Rasband at the National Institutes of Health, Bethesda, MD, United States and available at http://rsb.info.nih.gov/ij/). Hummingbird cells are characterized by the formation of needle-like projections[11]. We defined hummingbird phenotype as cells with needle-like projections > 15 μm. Uninfected AGS cells were used as a negative control.

Statistical analysis

Mann Whitney *U* test was used for statistical analysis. A *P* value < 0.05 was considered statistically significant. All data were analyzed with the software Graphpad Prism 5 (Graphpad Software, Inc.). All experiments were run in duplicates.

RESULTS

cagPAI status, CagA expression and tyrosine phosphorylation

From the 84 *cagA*-positive strains, 74 (88.1%) tested positive for *cagE,* 72 (85.7%) for *cagT* and 68 (81%) for *cag10*. Overall, 67 (79.8%) tested positive for all four *cag*PAI genes by PCR, and were therefore predicted, on the basis of this limited testing, to have an intact *cag*PAI. The remaining 17 strains, which tested negative for one or more genes, were collectively predicted to have a partial (*i.e.*, in-

Table 1 Primers used for the detection of *Cag*PAI genes

Gene	Primer	Sequence 5′-3′	Product size (bp)	Reference
cagE	101	TTGAAAACTTCAAGGATAGGATAGAGC	510	[16]
	102	GCCTAGCGTAATATCACCATTACCC		
cagT	cagTF	ATGAAAGTGAGAGCAAGTGT	823	[30]
	cagTR	TCACTTACCACTGAGCAAAC		
cag10	cag10F	ATGGAAGACTTTTTGTATAA	2208	[30]
	cag10R	TCACAGTTCGCTTGAACCCA		

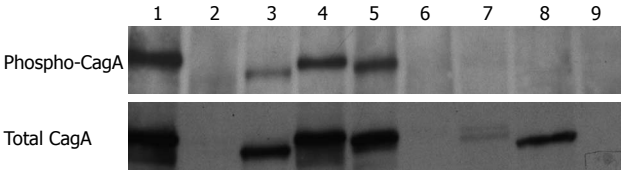

Figure 2 Phosphorylation of CagA protein in AGS cells after coculture with *Helicobacter pylori CagA*-positive strains. Cell lysates were evaluated by western blot using anti-phosphotyrosine or anti-CagA antibodies. A representative assay is shown. Lane 1: 11 637 control strain with a functional *cag*PAI and 5 Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs (ABCCC); Lanes 2 and 6: Isolates with a defective *cag*PAI lacking expression of CagA; Lanes 7 and 8: Isolates with a defective *cag*PAI expressing CagA, with absence of CagA phosphorylation; Lanes 3-5: Isolates with a functional *Cag*PAI with three (ABC; lane 3) or four (ABCC; lanes 4 and 5) EPIYA motifs; Lane 9: Uninfected AGS cells.

complete) *cag*PAI. Bacterial lysates of the 84 strains were assessed by Western blot with anti-CagA antibodies, from which 75 (89.3%) expressed the CagA protein. The nine strains lacking CagA expression harbored a partial *cag*PAI.

Once CagA is expressed, it is delivered into host cells *via* the T4SS and becomes phosphorylated by host cell kinases[11]. Seventy-three out of the seventy-five CagA-expressing strains were evaluated for CagA phosphorylation in coculture with AGS cells (Figure 2). From these, in 58 strains (79.4%) CagA was phosphorylated during infection. In 15 strains CagA was not phosphorylated, including eight CagA-expressing strains bearing a partial *cag*PAI. The remaining seven strains lacking CagA phosphorylation were predicted to have an "intact" *cag*PAI according to *cagT, cagE,* and *cag10* PCR results. This last result indicates that PCR detection of selected *cag*PAI genes is not sufficient to predict the functionality of the *cag*PAI. In addition, and as described below, these seven strains failed to induce IL-8 secretion in AGS cells, which supports this conclusion. Based on this *in vitro* characterization of the strains, we grouped isolates bearing a partial *cag*PAI (*i.e.*, strains which tested negative for one or more *cag*PAI genes) and strains with a non-functional *cag*PAI (*i.e.*, strains showing no CagA phosphorylation nor induction of IL-8 secretion) as strains with a defective *cag*PAI. In summary, 24 out of the 84 strains (28.5%) were found to harbor a defective *cag*PAI: 17 strains with a partial *cag*PAI and 7 strains with a non-functional *cag*PAI.

We further evaluated the association degree between the levels of CagA phosphorylation and the histopathological diagnoses for strains with functional *cag*PAI. Interestingly, the mean of CagA phosphorylation in strains from duodenal ulcer was shown to be significantly higher than the ones in strains from gastritis, atrophic gastritis, intestinal metaplasia and gastric cancer ($P < 0.02$, 0.045, 0.043 and 0.047 respectively; Figure 3A).

We also investigated the relationship between the number of EPIYA-C motifs and the levels of CagA phosphorylation. Isolates from this study ranged from one to three EPIYA-C motifs (Figure 1). CagA-expressing *H. pylori* strains bearing one and more than one EPIYA-C motifs were grouped together. Although higher levels of CagA phosphorylation were observed in strains with more than one EPIYA-C motif in comparison with strains with one EPIYA-C motif, this difference was not significant (Figure 3B).

Influence of cagPAI status and cagA polymorphisms on IL-8 expression in AGS cells

The 84 *cagA*-positive and 6 *cag*PAI-negative strains isolated from Colombian patients were tested for IL-8 induction in AGS cells. *CagA*-positive strains induced variable expression levels of IL-8 ranging from 0 to 810 pg/mL and from 8.8 to 1442 pg/mL at 6 h and 30 h post-infection, respectively. Ten out of the 67 strains classified by genotyping to bear an intact *cag*PAI did not induce IL-8 expression after 6 h of coculture with AGS cells. Three of these strains showed CagA translocation and phosphorylation in contrast to the remaining seven strains, in which no phosphorylation was observed. These last seven strains were likely to bear defects in other *cag*PAI components not detected by the PCR of the selected *cag*PAI genes. This showed, as previously observed by Argent *et al*[19], that PCR prediction of *cag*PAI intactness is a poor test for the presence of a T4SS capable of inducing IL-8 expression in AGS. We therefore considered these strains to have a non-functional *cag*PAI and classified them, along with the partial-*cag*PAI strains, as *cag*PAI-defective isolates, as described above.

*Cag*PAI-negative and *cag*PAI-defective strains did not induce detectable levels of IL-8 at 6 h post-infection (Figure 4A). At 30 h post-infection all strains induced IL-8 expression in AGS cells, although *cag*PAI-negative and *cag*PAI-defective strains induced significantly lower levels of IL-8 than strains with a functional *cag*PAI with one or more than one EPIYA-C motif ($P < 0.001$; Figure 4B).

A previous report has suggested a positive association between the number of EPIYA-C motifs and IL-8 expression[25]. We therefore evaluated *H. pylori*-IL-8 induction according to the number of EPIYA-C motifs in each strain. There were no differences in IL-8 expression

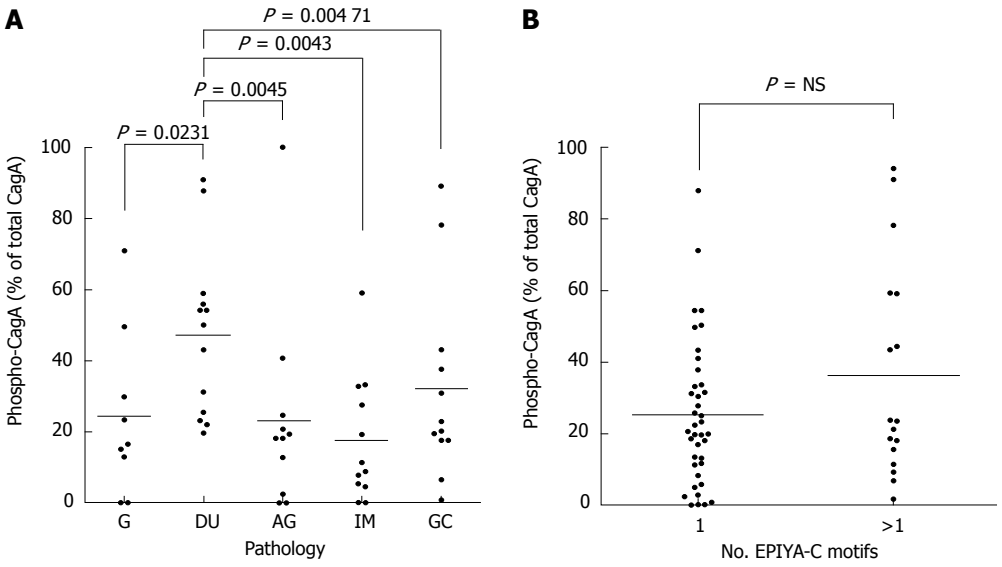

Figure 3 CagA-protein phosphorylation and its relationship to Glu-Pro-Ile-Tyr-Ala-C (EPIYA-C) motifs and disease severity. Sixty functional-*cag*PAI strains were cocultured with AGS cells for 6 h. Coculture lysates were assessed by Western blot and levels of CagA phosphorylation were determined by densitometry. A: Evaluation of CagA phosphorylation levels according to the pathology from which strains were isolated; B: Relationship between the number of EPIYA motifs and the levels of CagA phosphorylation. G: Gastritis; DU: Duodenal ulcer; AG: Atrophic gastritis; IM: Intestinal metaplasia; GC: Gastric cancer. NS: Not significant

among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 30 h of coculture, suggesting a lack of association between CagA EPIYA-C motifs variations in *H. pylori* isolates from Colombia and IL-8 induction (Figure 4A and B).

Influence of cagPAI status and cagA polymorphisms on hummingbird phenotype induction

AGS cells were cocultured with the same 84 strains tested for IL-8 expression and evaluated for hummingbird phenotype formation. We did not observe differences in the extent of cell elongation induction between strains with a functional or a defective *cag*PAI in 6 h cocultures (Figure 4C). At 24 h post infection strains with functional *cag*PAI showed high diversity in the extent of hummingbird phenotype induction ranging from 7% to 34%. *Cag*PAI-negative and *cag*PAI-defective strains induced significantly lower levels of elongation than strains with functional *cag*PAI with one or more than one EPIYA-C motif (P = 0.032 and 0.003 respectively; Figure 4D).

Similarly to IL-8 expression, no differences were observed in cellular elongation induction among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 24 h of coculture (Figure 4C and D). Unexpectedly, three *cag*PAI-defective strains induced elongation in more than 20% of the cells.

H. pylori-induced cellular responses and their association to disease severity

To assess the degree of association between the disease severity and IL-8 or cell elongation induction, strains with a functional *cag*PAI were grouped by the pathology from which they were isolated.

No differences were found in IL-8 expression among pathology groups, although small variations among IL-8 mean values were observed (Table 2). A slight increase in IL-8 mean values after 30 h of coculture in the direction Atrophic Gastritis, Intestinal Metaplasia, Gastric Cancer was observed. However, differences among groups were not significant. Interestingly, the two strains that showed the highest induction were isolated from patients diagnosed with gastric cancer.

Hummingbird phenotype induction had no significant association to disease severity either (Table 2). As in IL-8 induction, the two strains showing the highest cell elongation induction belonged to the gastric cancer group. However, these two strains were different from those inducing the highest IL-8 levels.

DISCUSSION

In Colombia, a country with high incidence of gastric cancer, 72% to 90% of *H. pylori* isolates harbor the *cagA* gene[31,32], a virulence factor associated with more severe disease[33]. It has been shown that the number of EPIYA-C motifs on CagA is associated with the levels of CagA tyrosine phosphorylation, SHP-2 binding activity and cytoskeletal alterations[17,26]. In this study we have evaluated the biological activities of *cagA*-positive Colombian strains on gastric epithelial cells according to the CagA polymorphisms, and their potential association with the severity of gastroduodenal diseases.

Although we included 84 *cagA*-positive strains in this study, the presence of this gene did not show strict concordance with the integrity of *cag*PAI, nor with the expression and delivery of CagA into epithelial AGS cells. About 28% of the strains were found to have a defective *cag*PAI, and 10%, in addition of being *cag*PAI-defective,

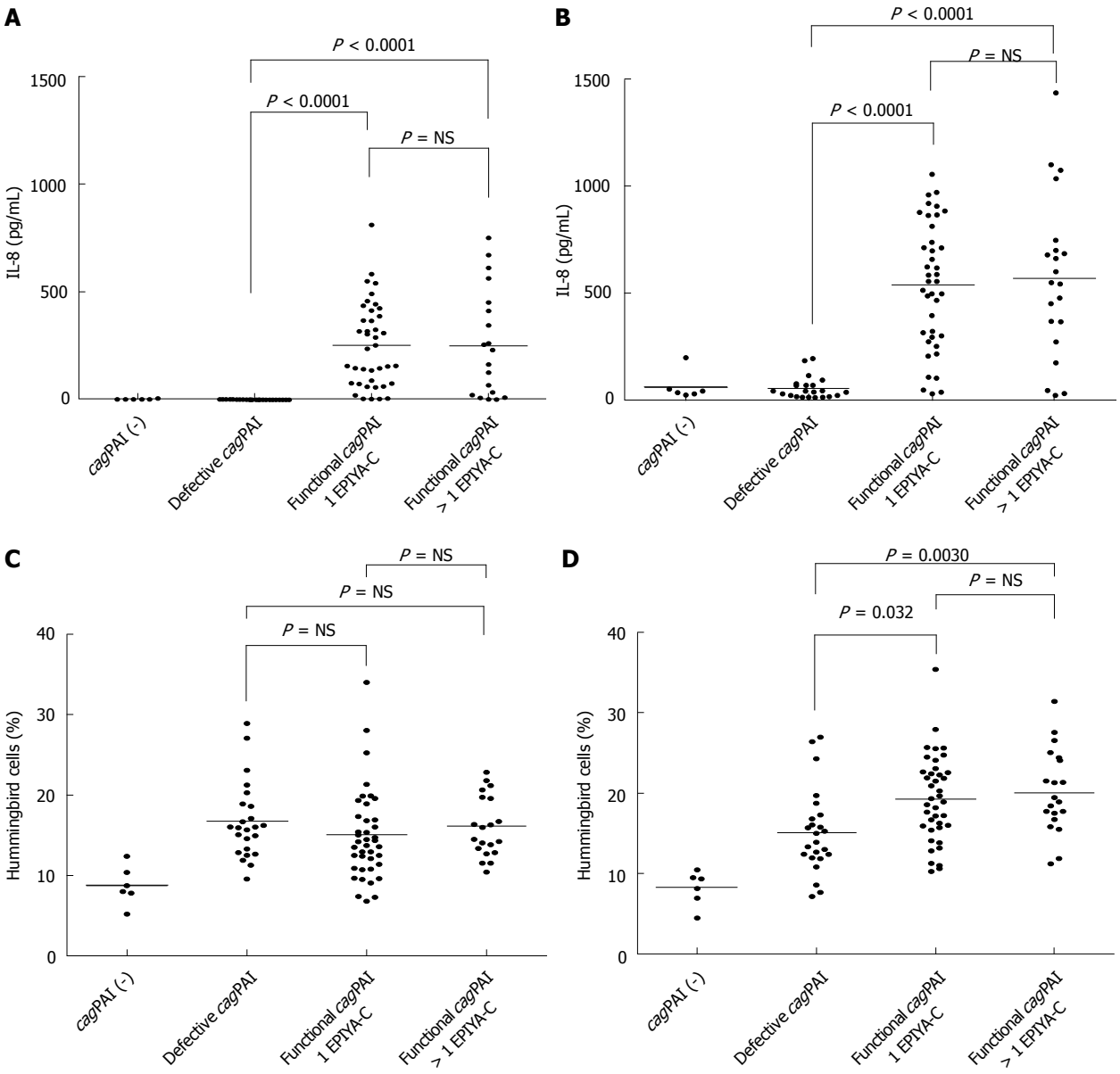

Figure 4 Influence of *cag*PAI status and CagA Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs variations on interleukin-8 expression and cell elongation. *Cag*PAI-negative strains (n = 6), *cag*PAI-defective strains (n = 24) and functional-*cag*PAI strains with either one (n = 40) or more than one (n = 20) EPIYA-C motifs were cocultured with AGS cells. (A) 6 h and (B) 30 h coculture supernatants were collected and assessed for interleukin-8 concentration by ELISA. Mean values are represented by horizontal lines within the scatterplots. Experiments for each strain were run in duplicates; (C) 6 h and (D) 24 h coculture photographs were taken and the percentage of hummingbird cells was determined. Mean values are represented by horizontal lines within the scatterplots. Experiments for each strain were run in duplicates. NS: Not significant.

Table 2 Induction of interleukin-8 expression and cell elongation by *cag*PAI-functional *Helicobacter pylori* strains according to the histopathological diagnosis

Pathology	IL-8 (pg/mL)				Elongation			
	6 h		30 h		6 h		24 h	
	mean	95%CI	mean	95%CI	mean	95%CI	mean	95%CI
Gastritis	256.1	(145.1-367.1)	594.5	(419.5-769.6)	15.3%	(10.7-19.9)	18.98%	(15.7-22.3)
Atrophic gastritis	224.7	(58.7-390.6)	463.7	(244.9-682.5)	14.9%	(12.2-17.6)	19.84%	(16.9-22.7)
Intestinal metaplasia	192.7	(89.0-296.4)	532.1	(398.2-666.0)	15.32%	(12.2-18.4)	17.7%	(18.5-20.6)
Gastric cancer	265.5	(107.5-423.5)	589.5	(355.4-823.5)	16.3%	(12.8-19.9)	19.55%	(15.9-23.1)
Duodenal ulcer	278.9	(154.8-402.9)	572.2	(343.6-800.8)	14.72%	(13.6-15.8)	19.8%	(17.2-22.4)

IL-8: Interleukin-8.

did not express the CagA protein. It is likely, that the promoter region of the *cagA* gene was disrupted in these strains, as previously reported for isolates from different human populations[34]. These results reinforce previous reports indicating that the presence of the *cagA* gene alone is not an accurate marker for an intact *cag*PAI[19,34-36].

Strains bearing an intact *cag*PAI showed high variability in CagA phosphorylation levels. *In vitro* experiments have shown that the number of EPIYA-C motifs is associated with the degree of CagA phosphorylation[26], and some studies with clinical isolates have also reported this association[19,27]. In our study we observed higher levels of CagA phosphorylation in strains with more than one EPIYA-C motifs than in strains with one EPIYA-C motif, although the differences were not significant. Considering that it has been proposed that CagA EPIYA motifs polymorphisms influence the degree of virulence as well as the oncogenic potential of individual *cagA*-positive strains[26], we evaluated the association between phosphorylation levels and histopathological diagnosis. We observed significant higher levels of CagA phosphorylation in strains from duodenal ulcer patients when compared to strains from the other pathologies, which is in agreement with a previous study reporting a similar behavior in strains isolated from duodenal ulcer patients[37]. It has been shown that *H. pylori*-induced inflammatory response is triggered upon CagA translocation into the host cell, where it activates NF-κB leading to IL-8 expression[12,13]. Furthermore, CagA-mediated IL-8 induction has been shown to be time- and strain-dependent. There is evidence demonstrating the importance of CagA for IL-8 expression in long incubation periods (24-48 h)[12,25]. However, the role of CagA in short incubation periods has been controversial. One study has found that isogenic *cagA*-mutant strains induce lower levels of IL-8 expression than their parental strains after 6 h and 9 h of co-culture[12]. In contrast, two studies, one involving isogenic *cagA* mutants and the second involving independent strains, found that IL-8 expression was not affected after 6 h of incubation[19,38]. Given these contradictory results, we tested IL-8 induction after 6 h and 30 h post-infection. We observed a clear CagA-dependent IL-8 expression pattern, as evidenced by the differences in IL-8 induction between *cagA*-negative/*cag*PAI-defective and *cag*PAI-functional strains. Strains bearing a functional *cag*PAI induced variable levels of IL-8 expression at 6 h of coculture, whereas *cagA*-negative and *cag*PAI-defective strains failed to induce IL-8 secretion. Furthermore, we confirmed the importance of CagA on IL-8 induction after long incubation periods, although we also detected low levels of IL-8 expression for *cagA*-negative and *cag*PAI-defective strains. These low levels of IL-8 induction at 30 h are probably the effect of other delayed responses like the CagA-independent IL-8 expression mechanism, in which *H. pylori* peptidoglycan translocated through bacterial membrane vesicles into epithelial cells activates, *via* Nod1, NF-κB resulting in IL-8 expression[39,40]. Furthermore, Crabtree *et al*[41] also reported low levels of secretion of IL-8 by *cagA*-negative strains after 24 h of infection. Taken together, our results support the concept of *H. pylori* time-dependent IL-8 induction, highlighting the importance of CagA for both, short and long, incubation periods.

We observed a lack of association between the number of EPIYA-C motifs and the level of IL-8 induction after coculture with AGS cells, even in prolonged incubation times. Neither an increasing number of EPIYA motifs nor an increasing number of EPIYA-C motifs had a boost effect on IL-8 expression. There are reports in the literature supporting our findings[42-44]. Reyes-Leon *et al*[43] reported no differences in IL-8 induction between Mexican *H. pylori* strains bearing one EPIYA-C motif and those with two or more C motifs. Interestingly, Mexican and Colombian *H. pylori* populations share common predominant polymorphisms (ABC and ABCC)[28,43,45]. Moreover, Sgouras *et al*[44] observed no differences in the levels of secreted IL-8 induced by individual isogenic subclones expressing CagA protein with different number of EPIYA-C motifs isolated from the same patient. It is worth noting the contrast of our results with those found by Argent *et al*[25], in which they observed a direct association between the number of EPIYA-C motifs and IL-8 expression in microevolved *H. pylori* strains from England. Discrepancies between studies may be explained by contrasting the geographical origin of the strains in each study. Our strains were isolated in Colombia and Argent strains were isolated in England, which are regions with high and mild gastric cancer risks, respectively[46]. It has recently been shown that *H. pylori* strains from different geographical and gastric cancer risk regions have distinct IL-8 induction behaviors in AGS cells[27]. Cellular inflammatory response in AGS cells was shown to be independent of the pathology from which strains were isolated, although the two strains showing the highest IL-8 expression levels were isolated from patients diagnosed with gastric cancer. These results are in agreement with previous studies showing that *H. pylori* strains isolated from different gastric pathologies varied in their ability to induce IL-8 expression in AGS cells, but did not associate to disease severity[19,47]. Moreover, Schneider *et al*[27] reported in a recent study involving *cagA*-positive *H. pylori* strains isolated from Colombian patients, that IL-8 expression induced by isolates from precancerous lesions did not differ from that induced by isolates from nonatrophic gastritis.

The induction of hummingbird phenotype upon infection with *cagA*-positive *H. pylori* strains has long been proposed as one of the mechanisms contributing to CagA oncogenic transformation[8,17]. Strains carrying biologically more active CagA have been associated with an increased risk of developing gastric carcinoma[17,19,22]. *H. pylori*-mediated cell elongation is potentiated by CagA proteins with higher number of EPIYA motifs[19,27]. Furthermore, proteins harboring higher number of EPIYA-C repeats increase hummingbird cells in AGS cells[17,26]. Our results disagree with these statements, as neither of both CagA molecular variations groups affected hummingbird

phenotype formation, but are in agreement with previous studies suggesting a lack of association between the number and type of EPIYA motifs and cellular elongation in *H. pylori* clinical isolates[22,37,43,48].

We found no differences in cell elongation induction when evaluating strains according to the pathology from which they were isolated. These results are in agreement with the results reported by Backert *et al*[37], in which strains isolated from German patients with different gastric pathologies showed no differences in cell elongation in AGS cells.

It is also important not to take *H. pylori* infection as the ultimate factor involved in gastric carcinogenesis, as there are many environmental and host factors associated with the disease. Polymorphisms in cytokine genes, such as *IL-8*, *IL-1β* and tumor necrosis factor-α, affect cytokine production upon *H. pylori* infection, increasing the risk of developing gastric diseases[2]. In addition to host genetic factors, environmental factors (*e.g.*, dietary and smoking habits) may also play an important role in *H. pylori* pathogenesis[49,50]. More interestingly, a recent study based on epidemiological and geographical data has proposed altitude as a surrogate for host, bacterial and environmental factors associated with gastric cancer risk[51].

In conclusion, we have reported a lack of association between *H. pylori* CagA protein EPIYA motifs variations from Colombian isolates and disease-associated cellular effects. Taken together, these results suggest that other factors (*e.g.*, host or environmental) may play a more important role than *H. pylori* CagA protein EPIYA variations in gastric cancer development in Colombia.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infect more than 50% of the world's population. Around 10%-15% of the infected individuals develop gastroduodenal diseases such as chronic gastritis, duodenal ulcers and gastric cancer. Currently, the determinants of the variable clinical outcomes have not been fully elucidated. One of the most important virulence factors of *H. pylori* is the *cag* pathogenicity island (*cag*PAI), which encodes several proteins, including CagA.

Research frontiers

CagA varies in size, and this variation has been shown to be due to Glu-Pro-Ile-Tyr-Ala (EPIYA) repeats within the C-terminal region of the protein. In Western *H. pylori* strains three types of EPIYA motifs have been described (A, B and C) based on the sequence flanking the motif. Strains with higher numbers of EPIYA-C motifs are more closely associated with gastric cancer and with an increased CagA *in vitro* activity, although this is controversial.

Innovations and breakthroughs

In contrast with studies in other populations, this study reports a lack of association between CagA EPIYA motifs variations from *H. pylori* colombian isolates and disease-associated cellular responses or gastroduodenal disease severity.

Applications

These results suggest that other factors (*e.g.*, host or environmental) may play a more important role than *H. pylori* CagA protein EPIYA variations in gastric cancer development in Colombia, a country with a high incidence of gastric cancer.

Terminology

H. pylori CagA protein is a virulence factor encoded in the *cag*PAI of the bacterium which is translocated into gastric epithelial cells through a type IV secretion system. Once in the cell, CagA becomes phosphorylated on tyrosine residues within the EPIYA motifs, mediating in turn the activation of several signaling pathways involved in the expression of pro-inflammatory cytokines such as interleukin-8 or in cell elongation, among others.

Peer review

The authors investigates the role of *cagA H. pylori* gene polymorphisms in the various bacterial-related chronic conditions. The paper is well designed and the results represent novel aspects of the infection consequences.

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BRIEF ARTICLE

Does in-house availability of multidisciplinary teams increase survival in upper gastrointestinal-cancer?

Christian Kersten, Milada Cvancarova, Svein Mjåland, Odd Mjåland

Christian Kersten, Milada Cvancarova, Department of Oncology, Southern Hospital Trust, 4604 Kristiansand, Norway
Svein Mjåland, the Centre for Cancer Treatment, Department of Oncology, Southern Hospital Trust, 4604 Kristiansand, Norway
Odd Mjåland, the Section of Abdominal Surgery, Department of Abdominal Surgery, Southern Hospital Trust, 4604 Kristiansand, Norway
Author contributions: All authors have contributed to the conception, design and interpretation of data and participated in drafting the manuscript, revised it critically and approved the final version; the detailed writing process and analysis of the data was performed by Cvancarova M and Kersten C.
Correspondence to: Christian Kersten, MD, PhD, Oncologic Consultant, Department of Oncology, Southern Hospital Trust, Postbox 416, 4604 Kristiansand, Norway. christian.kersten@sshf.no
Telephone: +47-913-39443 Fax: +47-381-46601
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Abstract

AIM: To investigate the effect of the establishment of in-house multidisciplinary team (MDT) availability (iMDTa) on survival in upper gastrointestinal cancer (UGI) patients.

METHODS: In 2001, a cancer centre with irradiation and chemotherapy facilities was established in the Norwegian county of West Agder with a change of iMDTa (WA/MDT-Change). "iMDTa"-status was defined according to the availability of the necessary specialists within one institution on one campus, serving the population of one county. We compared survival rates during 2000-2008 for UGI patients living in counties with (MDT-Yes), without (MDT-No), with a mix (MDT-Mix) and WA/MDT-Change. Survival was calculated with Kaplan-Meier method. Cox model was used to uncover differences between counties with different MDT status when adjusted for age, sex and stage.

RESULTS: We analyzed 395 patients from WA/MDT-Change and compared their survival to 12 135 UGI patients from four other Norwegian regions. Median overall survival for UGI patients in WA/MDT-Change increased from 129 to 300 d from 2000-2008, $P = 0.001$. The regions with the highest level of iMDTa achieved the largest decrease in risk of death for UGI cancers (compared to the county with MDT-Mix: MDT-Yes 11%, $P < 0.05$ and WA/MDT-Change 15%, $P < 0.05$). Analyzing the different tumour entities separately, patients living in the WA/MDT-Change county reached a statistically significant reduction in the risk of death [hazard ratios (HR)] compared to patients in the county with MDT-Mix for oesophageal and gastric, but not for pancreatic cancer. HR for the study period 2000-2004 are given first and then for the period 2005-2008: The HR for oesophageal cancers was reduced from [HR = 1.12; 95%CI: 0.75-1.68 to HR = 0.60, 95%CI: 0.38-0.95] and for gastric cancers from [HR = 0.87, 95%CI: 0.66-1.15 to HR = 0.63, 95%CI: 0.43-0.93], but not for pancreatic cancer [HR = 1.04-, 95%CI: 0.83-1.3 for 2000-2004 and HR = 1.01, 95%CI: 0.78-1.3 for 2005-2008]. UGI patients treated during the second study period in the county of WA/MDT-Change had a higher probability of receiving chemotherapy. In the first study period, only one out of 43 patients (2.4%, 95%CI: 0-6.9) received chemotherapy, compared to 18 of 42 patients diagnosed during 2005-2008 (42.9%, 95%CI: 28.0-57.8).

CONCLUSION: Introduction of iMDTa led to a two-fold increase of UGI patients, whereas no increase in survival was found in the MDT-No or MDT-Mix counties.

Key words: Gastric cancer; Gastroesophageal cancer; Oesophageal cancer; Pancreatic cancer; Multidisciplinary treatment; Multidisciplinary team; Survival

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INTRODUCTION

There is a lack of evidence that clinical decision making by a multidisciplinary team (MDT) leads to increased survival for oesophageal, gastric and pancreatic cancers[1-3].

The rationale for introducing MDTs is that modern cancer management has become increasingly complex, necessitating the involvement of various key professional groups in clinical decision making[4,5]. In addition, MDTs serve to monitor adherence to clinical guidelines and promote effective use of resources[1-3]. Evaluations of the effectiveness of a MDT on survival are warranted, but complicated to perform due to difficulties regarding: (1) its definition; (2) availability of valid measurement of its performance and, most importantly; and (3) the ethical and organizational hurdles of conducting prospective randomized studies of MDTs. Therefore, in this study we do not focus on the actual practice of MDTs but aim to analyse the effect of their in-house availability (iMDTa).

The most common upper-gastrointestinal (UGI) cancers in Norway are oesophageal, gastric and pancreatic[6]. Although these cancers have a dismal prognosis, the timely involvement of different medical specialists is advocated, based on a few studies on patients treated with curative intention[5,7-9]. However, these findings do not necessarily apply to palliative patients[10].

In 2001, a cancer centre with irradiation and chemotherapy facilities was established in the Norwegian county of West Agder with a change of iMDTa (WA/MDT-Change). Thus, iMDTa was established and the potential to work according to international MDT guidelines was created[4].

Our hypothesis was that the county of WA/MDT-Change, with its increasing iMDTa during the study period (from 2000-2008), would reach UGI cancer survival levels similar to those of a comparable Norwegian county with iMDTa. In addition, we hypothesized that the county of WA/MDT-Change would have favourable UGI cancer survival figures compared to a county without iMDTa during said period.

The primary objective was to evaluate the effect of the establishment of iMDTa on overall survival in a cohort of UGI cancer patients living in the Norwegian county of WA/MDT-Change.

MATERIALS AND METHODS

For detailed information about Norway's health care system, the Cause of Death Registry, The Cancer Registry, the establishment of MDT-availability and the actual performance of MDT in the county of WA/MDT-Change, as well as a description of the other counties included for comparison, see electronic supplement. A short overview is given below.

Norway

Norway is a country with very little migration or socio-economic disparity[11,12], amongst its 5 million inhabitants (Table 1). Health care coverage in Norway is provided through a single-payer universal government funded system. All persons residing in Norway are assigned a unique 11-digit identification number, making it possible to link information from various national registries.

Cause of Death Registry

Physicians are required by law to complete a death certificate for all deaths in Norway. The Cause of Death Registry[13] collects all death certificates for coding and registration of the cause of death.

The Cancer Registry

The Cancer Registry of Norway[6] (CRN), has collected data on all cancers that have occurred in Norway since 1953. Medical doctors are required by law to report these diagnoses, ensuring high levels of completeness[14]. Cancer type, date of diagnosis, extent or stage of the disease at diagnosis, and initial treatment in broad terms, are recorded.

Changes in WA/MDT-Change during 2000-2008

The Sørlandet Hospital Trust is the regional hospital in the county of WA/MDT-Change, which serves a stable population of approximately 170 000 inhabitants. In 2001, the Centre for Cancer Treatment was established at this hospital, thereby creating the potential for in-house MDTs. During the study period, the number of oncologists has increased from one consultant one day every fourth week to six full-time oncologists and two house officers. Prior to the establishment of the cancer centre, patients had to be referred for irradiation or complex chemotherapy to Oslo University Hospital, 300-500 km (a four to six hour drive) away.

In the ensuing years, increasing oncologic and palliative care expertise has developed and was practiced in conjunction with the already well-established pathological, radiological, gastrosurgical and gastroenterological specialties. Specifically, the following services were founded: A mobile palliative care team in 2002, an outpatient palliative care day centre in 2004 and an in-patient palliative care unit with ten beds in 2007. Prior to 2005, the management of cancer patients across specialties was discussed in informal and undocumented encounters between practitioners. From the summer of 2005 and onwards however, weekly MDT-meetings with a designated focus on gastrointestinal cancers have been held, with gastroenterologists, gastrointestinal surgeons, radiologists and oncologists present.

Other analyzed regions

Throughout the study period the inhabitants of the analysed regions selected for comparison had the same life expectancies and very similar socioeconomic conditions. Further details can be found in the electronic supplement (Table 1).

The choice of the Norwegian counties used for comparison to WA/MDT-Change in this study, is based on

Table 1 Cox proportional hazards model adjusted for age and the different regions

		MDT-No	MDT-Change	MDT-Yes	Rest of Norway
All UGI cancers	2000-2004	HR 0.96, (0.83-1.10)	HR 0.96, (0.82-1.13)	HR 0.90[1], (0.80-1.0)	HR 0.96, (0.83-1.11)
	2005-2008	HR 0.96, (0.82-1.13)	HR 0.81[1], (0.67-0.97)	HR 0.79[1], (0.70-0.89)	HR 0.85[1], (0.77-0.93)
Oesophagus	2000-2004	HR 0.75, (0.49-1.15)	HR 1.12, (0.75-1.68)	HR 0.86, (0.63-1.17)	HR 0.89, (0.73-1.1)
	2005-2008	HR 1.08, (0.72-1.61)	HR 0.60[1], (0.38-0.95)	HR 0.74[1], (0.53-1.02)	HR 0.84, (0.67-1.06)
Gastric	2000-2004	HR 0.94, (0.70-1.26)	HR 0.87 (0.66-1.15)	HR 0.99, (0.84-1.12)	HR 0.94, (0.70-1.25)
	2005-2008	HR 0.94, (0.70-1.23)	HR 0.63[1], (0.43-0.93)	HR 0.79[1], (0.65-0.97)	HR 0.82[1], (0.70-0.95)
Pancreas	2000-2004	HR 0.97, (0.79-1.2)	HR 1.04, (0.83-1.3)	HR 0.90, (0.77-1.06)	HR 1.02, (1.02-1.03)
	2005-2008	HR 0.92, (0.74-1.2)	HR 1.01, (0.78-1.3)	HR 0.84[1], (0.71-1.0)	HR 0.88, (0.78-1.0)

UGI: Upper gastrointestinal cancer; MDT: Multidisciplinary team. Hazard ratios (HR) are given with 95% confidence interval, and the county of Oslo with MDT-Mixed serves as reference. [1]Statistically significant with *P*-value < 0.05.

their stable status of iMDTa during the study period.

In this manuscript, we define "iMDTa" as a county's theoretical possibility of MDT meetings within a single administrative institution with all departments on one campus (MDT-Yes). Thus, we measured the possibility of multidisciplinary in-house cooperation of necessary specialists, rather than the formal performance of MDTs. A county with stable iMDTa during the entire study period (MDT-Yes) was hypothesized to have the best survival figures for UGI patients. MDT-No describes a county with an absence of radiation units and medical oncologists within the hospital, where patients were referred to a tertiary university hospital for oncologic treatment during the entire study period. However, gastrointestinal surgeons, gastroenterologists, radiologists and, pathologists were available in such county. The population of the county of Oslo was treated partly in hospitals with all these services available and partly in hospitals without some of these services in the same institution. Therefore this region was defined as MDT-Mixed.

Patients

For the county of WA/MDT-Change, we used the hospital's electronic database and confirmed and supplemented it with data from CRN. Further, we identified patients diagnosed with oesophageal, gastric or pancreatic cancers during the study period. Only patients with adenocarcinomas and squamous cell carcinomas were included. The clinical course of the disease of each patient was reviewed. Data regarding oncological, surgical and endoscopic interventions were collected. If surgery had been performed, it was characterized as curative or palliative. Survival figures between different regions were compared using data from CRN.

Ethics

The study was approved by the Regional Ethics Committee of Southern Norway. The anonymity of the patients included in the analysis was preserved according to the institutional guidelines of our hospital as well as those of the National Data Protection Commission of Norway.

Statistical analysis

Complete follow-up data were available on all patients. They were followed from the date of diagnosis to their death or the date of censoring (July 2011). Crude survival was calculated using the Kaplan-Meier method. Crude differences in survival were assessed with log-rank test. Further, to adjust for possible confounding multivariate Cox regression models were fitted. All models were adjusted for age, sex, stage and region and fitted separately for the two diagnostic periods. The results were presented as hazard ratios (HR) with 95% confidence intervals (CI). When assessing the regional differences, the county with MDT-Mix was used as a reference because its population was the largest (to ensure stability of the estimates). *P*-values of less than 0.05 were considered statistically significant. All analyses were performed with SPSS and Stata.

RESULTS

Patients

The annual incidences of oesophageal, gastric and pancreatic cancers in Norway from 2004 through 2008 were 4.1/100 000, 11.1/100 000, and 13.6/100 000, respectively. We analyzed 12 530 UGI patients living in five Norwegian regions, there of 395 patients in the county of WA/MDT-C. Median age at diagnosis was 74 years (17-98 years) and median follow-up was 5 mo (0-138 mo).

The baseline characteristics of the patients are listed in Table 2.

No clinically relevant differences in stage distribution of UGI cancers were revealed among the analyzed regions or between the two calendar periods. Furthermore, the stage distribution remained stable during the whole study period. Roughly 40% of all UGI cancer patients had distant metastases at the time of their diagnosis.

The changes in survival over time are illustrated with Kaplan-Meier curves in Figure 1.

During the study period,the largest increases in survival were seen in the county of WA/MDT-Change, see green curve in Figure 1. Here, median survival for oesophageal cancer patients increased from 5 mo (3-12 mo) to 11 mo (9-23 mo) and from 7 mo (4-12 mo) to 15 mo (4-35 mo) for gastric cancer patients. However, these increases were not statistically significant. This numerical survival gain could not be observed in the MDT-No county (red curve) or in the MDT-Mix county (blue

Table 2 Patient characteristics *n*(%)

	Diagnosed Jan 2000-Dec 2004					Diagnosed Jan 2005-Dec 2008				
	MDT-Mix	MDT-No	MDT-Change	MDT-Yes	Other regions	MDT-Mix	MDT-No	MDT-Change	MDT-Yes	Other regions
Tumor type										
Oesophagus	113 (15.4)	31 (11.8)	30 (14.6)	68 (10.0)	657 (12.9)	91 (15.2)	35 (16.2)	30 (19.6)	72 (12.8)	571 (14.1)
Gastric	258 (35.2)	110 (41.8)	77 (37.6)	318 (47.0)	2146 (42.2)	214 (35.8)	73 (33.8)	45 (29.4)	240 (42.6)	1534 (38.0)
Pancreas	362 (49.4)	122 (46.4)	98 (47.8)	291 (43.0)	2278 (44.8)	293 (49.0)	108 (50.0)	78 (51.0)	252 (44.7)	1935 (47.9)
Total	733 (100.0)	263 (100.0)	205 (100.0)	677 (100.0)	5081 (100.0)	598 (100.0)	216 (100.0)	153 (100.0)	564 (100.0)	4040 (100.0)
Stage										
No metastasis	109 (14.9)	38 (14.4)	28 (13.7)	81 (12.0)	694 (13.7)	59 (9.9)	30 (13.9)	28 (18.3)	71 (12.6)	543 (13.4)
Lymph node metastasis	155 (21.1)	62 (23.6)	49 (23.9)	182 (26.9)	1195 (23.5)	125 (20.9)	52 (24.1)	44 (28.8)	141 (25.0)	926 (22.9)
Distant metastasis	293 (40.0)	104 (39.5)	85 (41.5)	303 (44.8)	2083 (41.0)	237 (39.6)	97 (44.9)	57 (37.3)	237 (42.0)	1642 (40.6)
Unknown	176 (24.0)	59 (22.4)	43 (21.0)	111 (16.4)	1109 (21.8)	177 (29.6)	37 (17.1)	24 (15.7)	115 (20.4)	929 (23.0)
Total	733 (100.0)	263 (100.0)	205 (100.0)	677 (100.0)	5081 (100.0)	598 (100.0)	216 (100.0)	153 (100.0)	564 (100.0)	4040 (100.0)

MDT: Multidisciplinary team. No clinically relevant differences in stage distribution were revealed among the analyzed regions or between the two calendar periods. In addition, there were no clinically relevant differences in stage distribution among the studied counties.

curve), whereas a survival gain could be observed in the MDT-Yes county (yellow curve).

After analyzing crude survival, survival was adjusted for age, region, sex and stage (even though no differences regarding sex and stage were found among the analysed regions). Comparing the two calendar periods of 2000-2004 and 2005-2008, the regions with the highest level of iMDTa achieved the largest decrease in risk of death for all UGI cancers (Table 1, compared to the county with MDT-Mix: MDT-Yes 11% and WA/MDT-Change 15%).

Analyzing the different tumour entities separately, the WA/MDT-Change county reached a statistically significant reduction in the risk of death (HR) compared to the county with MDT-Mix for oesophageal and gastric, but not for pancreatic cancer. HR for the study period 2000-2004 are given first and then for the period 2005-2008: The HR for oesophageal cancers was reduced from [HR = 1.12, 95%CI: 0.75-1.68 to HR = 0.60, 95%CI: 0.38-0.95] and for gastric cancers from [HR = 0.87, 95%CI: 0.66-1.15 to HR = 0.63, 95%CI: 0.43-0.93], but not for pancreatic cancer [HR = 1.04-, 95%CI: 0.83-1.3 for 2000-2004 and HR =1.01, 95%CI: 0.78-1.3 for 2005-2008].

Treatment and survival changes in the county of WA/MDT-Change

Hospital records for the region with changing status of iMDTa were analyzed to confirm the UGI cancer incidence numbers from the national registries, as well as the gain in survival. Further, we searched for changes in use of potentionall potentially life-prolonging oncologic interventions for the county WA/MDT-Change.

A total of 395 patients with UGI cancers were identified in the hospital records of the WA/MDT-Change county. These data are in full accordance with the incidence figures estimated by The National Cancer Registry[6].

The survival for all UGI cancer patients in the WA/MDT-Change county increased especially after 2004, when MDT meetings became more formalized. Median overall survival for all MDT-Change UGI cancer patients increased significantly from 129 d in the year 2000 to 300 d in 2008, $P = 0.001$. Also these data were in accordance with figures from CRN[6].

During the study period, several organizational changes were made at the Sørlandet Hospital Trust in the county of WA/MDT-Change. In line with the increased iMDTa, changes in the rates of curative surgery, oesophageal or bile duct stent placement, irradiation or chemotherapy were likely to have occurred and these were therefore analyzed.

UGI patients treated during the second study period had a higher probability of receiving chemotherapy. In the first study period, only one out of 43 patients in WA/MDT-Change (2.4%, 95%CI: 0-6.9) received chemotherapy, compared to 18 of 42 patients diagnosed during 2005-2008 (42.9%, 95%CI: 28.0-57.8).

The number of irradiation series did not increase for the diagnoses in question (data not shown).

During the study period, no major changes in surgical practice took place and there was no statistically significant increase in the number of curative UGI cancer surgeries (data not shown). No statistically significant increase in the use of gastro oesophageal or bile duct stents was observed during the two calendar periods of 2000-2004 and 2005-2008 (data not shown).

DISCUSSION

In this study, we found a more than two-fold increase in median survival for UGI cancer patients living in a Norwegian county during a time period in which in-house MDT has become available there. This increase in survival was not observed in counties without full iMDTa, but we saw a survival gain in both counties with iMDTa (MDT-Yes and MDT-Change).

The results of the described organizational changes are striking and clinically relevant, particularly in light of the limited advances in medical treatment of UGI cancer

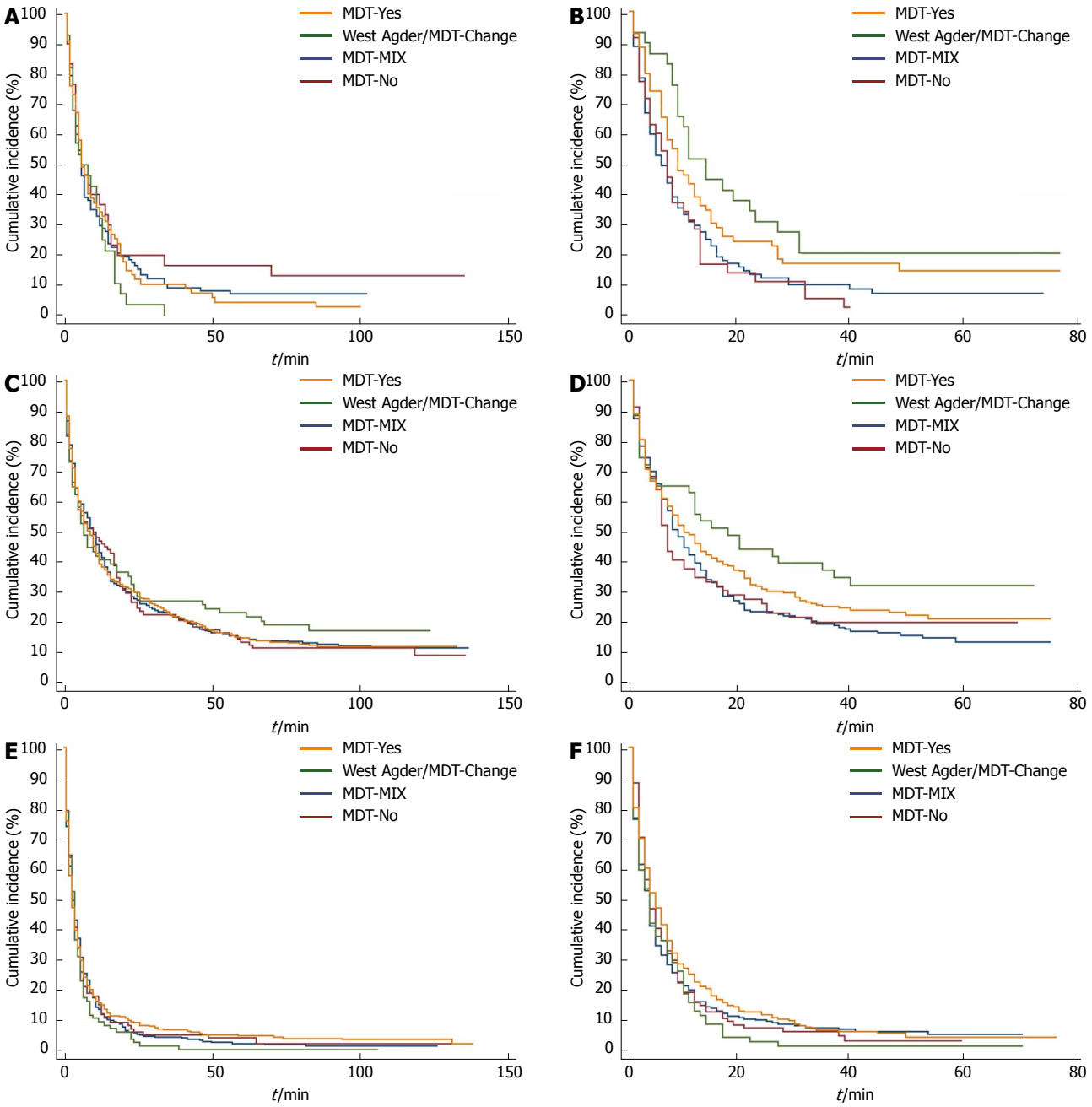

Figure 1 Increase in survival in counties with high in-house multidisciplinary team-availability during the second study period. A, B: Oesophagus; C, D: Stomach; E, F: Pancreas; A, C, E: 2000-2004; B, D, F: 2005-2008. MDT: Multidisciplinary team.

patients during the same time period[15].

This study is one of very few, that report a survival benefit of MDTs in cancer care. MDT meetings require a considerable amount of time from core specialists. Therefore, the need to confirm MDTs' effectiveness on survival is of increasing importance, since there is an accelerating shortage of professional groups required for MDTs[16].

A major strength of the present study is its unique setting. Typically, before-after series[1-3] are confounded by concurrent changes in other factors, such as better treatments or different stage mixes, over the studied time period. In Norway, relatively few and stable socioeconomic differences are combined with an egalitarian public health service. In addition, high quality national cancer and death registries have been established decades ago. Furthermore, life expectancies were stable and similar in the analyzed regions throughout the study period. We were therefore able to analyze the un-confounded effect of changes in the organization of health care on survival of selected patient groups living in different regions.

Most importantly, survival outcomes can be attributed to patients' residence, even if a few of them were operated or irradiated in other regions, thus indicating the quality of health care provided for the population living in a defined region. In addition, we have compared patient

survival among regions between two time periods which were consequitive. Therefore, it is unlikely that significant changes in the possible confounding factors over a time period of 3-4 took place.

The precise role and composition of MDTs in cancer care vary throughout the world. Moreover, these variations exist even from hospital to hospital within the same region or country. Further hurdles in MDT research are the different interpretation of MDT-guidelines and the validity of documentation of the actual performance according to these guidelines[2]. Moreover, we are just starting to understand the individual factors of MDTs affecting the clinical outcome[17]. In the county of WA/MDT-Change, the MDTs were organized in line with international MDT guidelines[4], and aimed to perform accordingly.

While using registry data for patient identification prevents bias associated with clinician selection of patients, registry retrieved data has limitations with respect to the variables available for analysis. In addition to the CRN, we had complete hospital records for the region with changing status of iMDTa and could therefore analyze the changes in use of potentially life-prolonging oncologic interventions for the MDT-Change county. One measureable factor potentially contributing to the increase in survival in WA/MDT-Change may be the increased use of chemotherapy. This increase is higher than expected for this time period. A 50% increase in the use of chemotherapy for every year of the study period may be a result of more patients getting therapy. In that respect, MDT seems to result in increased referral of UGI patients to the medical oncologist. Travel distance to hospitals has been shown by others to be a barrier to treatment among patients with most types of cancer, including UGI cancers[18-20]. Furthermore, the EUROCARE working group found striking differences in gastric cancer survival and the quality of management logistics has been proposed as an important variable for patient survival[21]. In line with this argument, gastric cancer patients at district hospitals more often received adjuvant chemotherapy, than patients treated in university hospitals in Norway[22]. Unfortunately, we have not been able to analyze to what extent patients in other regions had received chemotherapy. However, in light of the striking survival gains, it appears that the increasing use of chemotherapy is unlikely to be the only reason for the survival gain seen in WA/MDT-Change.

The number of irradiation series or the use of gastro oesophageal or bile duct stents did not increase during the study period and we do not consider that the changes of the palliative services had a major impact on the life expectancy of the study patients, since the in-patient service was established at the very end of the study period.

The role of surgery for survival of the whole study cohort in this setting is more complex, since the group of UGI cancers as a whole has a low rate of curative surgery. Pancreatic and esophageal cancers are operable in less than one of five cases[15], and small changes in this ratio affect median overall survival to a limited extent. Concerning gastric cancer, 43% of cases are operated in Norway[22]. This proportion was allready higher before (data not shown), but stable throughout the study period, in our clinic. Thus, in light of a relatively high rate of surgery before iMDTa, the rate of surgery was not affected through the establishment of iMDTa in the WA/MDT-Change county. When looking at the results for gastric and esophageal cancers (Table 2), two findings are interesting: Both MDT-No and WA/MDT-Change centralized surgical treatment of esophageal, but not gastric cancer during the second interval of the study period to the MDT-Mix county of Oslo. In the WA/MDT-Change county, a survival benefit was seen for both entities, whereas the survival for these two diagnoses decreased in the MDT-No county (Table 2). These findings may support the theory that, in these particular geographic regions, the presence of oncologists in a hospital may have a greater impact than the place of curative surgery, at least on short term survival.

In this respect, the increased use of chemotherapy should be interpreted as an effect modifier for survival and the MDT members in WA/MDT-Change agree upon a during the study period gradually improved team spirit and more effective communication, although it seems easier to measure the results, rather than formally proof the process of such increased human interdependency.

A limitation of our study is the relatively low incidence of UGI cancers. We therefore analysed survival changes for all stages combined for each cancer type. As the main goal of our study was to assess changes in survival for the entire group of UGI cancer patients, we consider our results valid because the stage distribution for a given diagnosis in the different regions did not change during the course of the study period. In addition, the limited life expectancy for UGI cancer patients makes it possible to compare and assess results concerning improved patient outcome after treatment changes in a way that cannot be achieved in entities with long term survival. Seventy percent of UGI cancer patients live shorter than one year, making short term survival figures important both for the patients and health care administrators when considering organizational changes.

The explanation of the increased survival seen after the introduction of in-house MDT-availability is most likely multi-factorial. Future prospective studies should also analyze the communicative implications at play when a team is formed in-house over a period of several years and assessment tools for this purpose have been created[23]. Most importantly, it is not clear if the present findings for UGI cancer patients can be extrapolated to other cancer entities. This question should be addressed in future research.

In conclusion, we present one of the first studies showing a survival benefit for oesophageal and gastric cancer patients after the establishment of MDTs. We found a striking and more than two-fold increase in survival among patients with UGI cancers living in a Norwegian county with increasing iMDTa. During the analysed time period,

no increase in survival was found in counties without consistent MDT availability. The survival gain might be partly explained by increased use of chemotherapy.

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COMMENTS

Background

There is a lack of evidence that clinical decision making by a multidisciplinary team (MDT) leads to increased survival for oesophageal, gastric and pancreatic cancers

Research frontiers

The rationale for introducing MDTs is that modern cancer management has become increasingly complex, necessitating the involvement of various key professional groups in clinical decision making. In addition, MDTs serve to monitor adherence to clinical guidelines and promote effective use of resources. Evaluations of the effectiveness of a MDT on survival are warranted, but complicated to perform due to difficulties regarding: (1) its definition; (2) availability of valid measurement of its performance and, most importantly; and (3) the ethical and organizational hurdles of conducting prospective randomized studies of MDTs.

Innovations and breakthroughs

In this study, authors did not focus on the actual practice of MDTs but aim to analyse the effect of their in-house availability. This is the first study to document a survival benefit of upper gastrointestinal (UGI) cancers after the implementation of MDT.

Applications

This study gives evidence to the wideheld belief of a survival benefit in UGI cancer patients, when treated in a setting of MDT.

Terminology

Here, the term "in-house MDT" was introduced and efined as a county's theoretical possibility of MDT meetings within a single administrative institution with all departments on one campus (MDT-Yes).

Peer review

The authors examined the survival benefit of UGI cancer patients after the introduction of in-house MDT and compared the survival to the geographic regions with and without in-house MDT.

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CASE REPORT

Gastroesophageal cancer and retroperitoneal fibrosis: Two case reports and review of the literature

Renata D'Alpino Peixoto, Jasem Al-Barrak, Howard Lim, Daniel Renouf

Renata D'Alpino Peixoto, Jasem Al-Barrak, Howard Lim, Daniel Renouf, Department of Medical Oncology, BC Cancer Agency, Vancouver, BC V5Z 4E6, Canada

Authors contributions: Peixoto RD, Lim H and Renouf D designed the report; Lim H and Renouf D were attending doctors for the patients; Peixoto RD, Al-Barrak J, Lim H and Renouf D organized the report; Peixoto RD, Al-Barrak J, Lim H and Renouf D wrote paper.

Correspondence to: Renata D'Alpino Peixoto, MD, Department of Medical Oncology, BC Cancer Agency, 600 West 10th Avenue, Vancouver, BC V5Z 4E6, Canada. renatadalpino@gmail.com

Telephone: +1-604-8776000 Fax: +1-604-8770585

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Abstract

Retroperitoneal fibrosis secondary to malignant disease is a rare condition associated with a dismal prognosis. We herein present the first ever reported case of retroperitoneal fibrosis related to esophageal adenocarcinoma in a 63-year-old patient who developed bilateral ureteral obstruction due to extensive retroperitoneal fibrosis 18 mo after having completed neoadjuvant chemoradation followed by surgery for a pT3N0 adenocarcinoma of the distal esophagus. We also report the case of a previously healthy woman who presented with bilateral ureteral obstruction and diffuse narrowing of the common biliary duct and was found to have extensive retroperitoneal fibrosis as a consequence of metastatic gastric adenocarcinoma. Both patients had poor performance status and were unsuitable for palliative chemotherapy. This paper shows that urinary and biliary obstructive symptoms might represent retroperitoneal fibrosis as a consequence of gastroesophageal malignancy.

Key words: Gastric cancer; Esophageal cancer; Retroperitoneal fibrosis

Peixoto RD, Al-Barrak J, Lim H, Renouf D. Gastroesophageal cancer and retroperitoneal fibrosis: Two case reports and review of the literature. *World J Gastrointest Oncol* 2013; 5(3): 68-70 Available from: URL: http://www.wjgnet.com/1948-5204/full/v5/i3/68.htm DOI: http://dx.doi.org/10.4251/wjgo.v5.i3.68

INTRODUCTION

Retroperitoneal fibrosis is a rare clinical condition characterized by the presence of pathologic collagen plaque around the abdominal aorta and iliac vessels, as well as the inferior vena cava and the ureters. Approximately 70% of retroperitoneal fibrosis is idiopathic in nature, while the remaining 30% are believed to be related to certain drugs (ergot-derivatives, methysergide, bromocriptine, beta-blockers, methyldopa, analgesics, hydralazine), malignancy (carcinoid, lymphoma, sarcoma, carcinomas of the colon, prostate, breast, stomach), infections (tuberculosis, histoplasmosis, actinomycosis), radiotherapy (testicular seminoma, colon carcinoma, pancreatic carcinoma), surgery (lymphadenectomy, colectomy, hysterectomy, aortic aneurysmectomy), trauma, amyloidosis[1,2]. From our knowledge, there are only nine reported cases of retroperitoneal fibrosis associated with gastric cancer[3-11], while there is no report associated with esophageal cancer.

CASE REPORT

Case 1

A 63 year-old man with a past history of pT3N0 adenocarcinoma of the distal esophagus treated with neoadjuvant chemoradiation (5 wk of chemoradiation at dose 50.4 Gy with cisplatin 25 mg/m^2 days 1-3 and 5-fluorouracil 1000 mg/m^2 daily × 4, weeks 1 and 5) followed

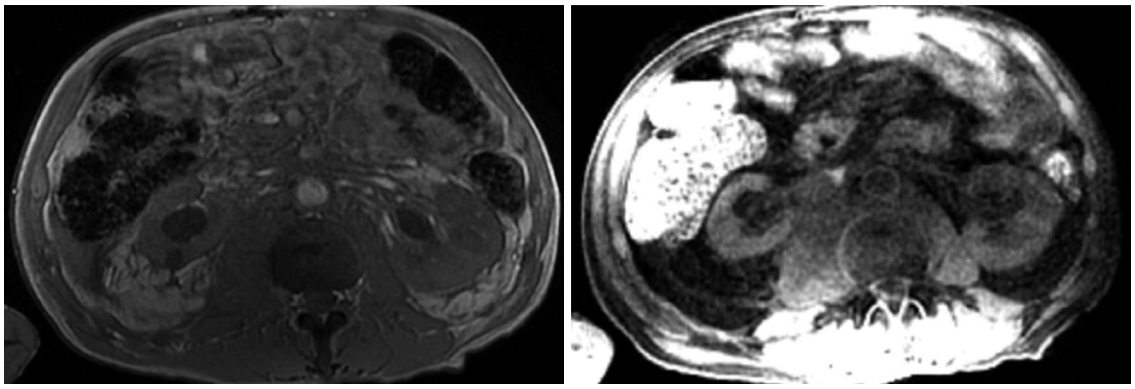

Figure 1 Magnetic resonance imaging of the abdomen revealing an ill-defined retroperitoneal infiltrate.

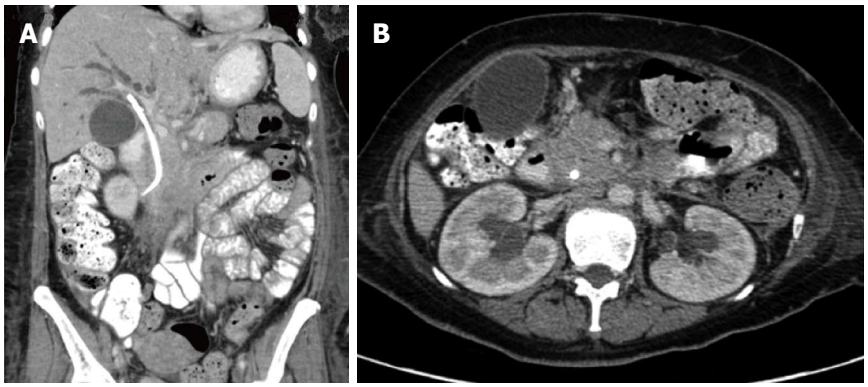

Figure 2 Computed tomography scan of the abdomen revealing an extensive retroperitoneal soft tissue mass. A: Sagittal view; B: Transverse view.

two-hole esophagectomy with gastric reconstruction presented 18 mo after the completion of treatment with acute kidney injury caused by bilateral ureteral obstruction. Bilateral nephrostomy tubes were placed. Magnetic resonance imaging of the abdomen revealed an ill-defined retroperitoneal infiltrate extending from the level of the renal vessels towards the presacral space distally demonstrating retroperitoneal fibrosis (Figure 1). The patient underwent a biopsy of the retroperitoneum which showed poorly differentiated adenocarcinoma consistent with metastasis from the previous esophageal cancer.

Case 2

A previously healthy 54-year-old woman who presented to the emergency department with a 4-wk history of flank pain, persistent nausea with vomiting, anorexia and progressive oliguria as well as intermittent hematuria. The patient was found to have bilateral hydronephrosis due to ureteral obstruction with consequent renal insufficiency and underwent bilateral nephrostomy. Shortly after admission, she also became jaundiced. Subsequent work-up revealed diffuse narrowing of the common biliary duct and a biliary stent was inserted. A liver biopsy was consistent with cholestasis and immunoglobulin G4 level was normal. Computed tomography (CT) scan of the abdomen showed extensive retroperitoneal soft tissue mass, extending all the way up to the liver hilum as well as diffusely narrowed caliber of the inferior vena cava and portal vein (Figure 2). These findings were consistent with retroperitoneal fibrosis. During an attempt to an endoscopic-ultrasound guided fine-needle aspiration of the retroperitoneum mass, she was found to have thickening of the gastric wall. Gastric biopsy revealed invasive adenocarcinoma in a scenario of linitis plastica while the ascetic fluid revealed malignant cells.

DISCUSSION

Symptoms caused by this fibrotic process are usually secondary to compression and constriction of local anatomic structures. The most frequent presenting symptom is pain in the lower back, flank or abdomen, which tends to increase over time[12]. Other common symptoms include weight loss, anorexia, testicular pain, edema, and gross hematuria[2]. In late stages, patients may develop progressive ureteral obstruction with renal insufficiency due to encasement of both ureters by the retroperitoneal mass. More rarely, involvement of the biliary tree by the fibrotic tissue may cause obstructive jaundice[13], as was the case of our second patient.

In most cases of retroperitoneal fibrosis secondary to malignant disease, abnormal collagen plaque in the retroperitoneum results from an exuberant desmoplastic response to retroperitoneal metastases[2]. It is believed to be an immune-mediated process, in which macrophages release cytokines that stimulate fibroblast proliferation with subsequent fibrosis. However, its etiology and pathobiology remain obscure. This mechanism is different in car-

conoid tumors, which may lead to retroperitoneal fibrosis without the presence of metastasis probably through a serotonin-mediated mechanism[14]. Another possible explanation for carcinoid-induced retroperitoneal fibrosis is the release of profibrogenic growth factors such as platelet-derived growth factor, insulin-like growth factors, epidermal growth factor, and the family of transforming growth factors α and β[15].

The diagnosis of retroperitoneal fibrosis is primarily made by imaging studies. Contrast-enhanced CT scan is the method of choice as it visualizes the extent of fibrosis and may assess the presence of metastatic tumor. Moreover, CT scan may also enable CT-guided biopsy[16]. Positron emission tomography (PET)-CT has recently been reported as a useful imaging modality in idiopathic retroperitoneal fibrosis, not only for diagnosis but also for treatment response evaluation[17]. Because retroperitoneal fibrosis is a metabolically active tissue, it will show increased radiotracer uptake, irrespective of a malignant or idiopathic cause. However, PET-CT scan may reveal an occult primary tumor as well as metastatic disease. Biopsy of the retroperitoneum is highly recommended if there is suspicion for an underling malignancy.

Usually retroperitoneal fibrosis secondary to malignant disease is associated with a dismal prognosis. The nonspecific symptoms often make the diagnosis very difficult and during late stages patients may have organ dysfunction and poor performance status, being unsuitable for palliative chemotherapy. Both of our patients were not able to undergo chemotherapy. Unfortunately, there is no evidence in the literature that chemotherapy would help reducing malignancy-related retroperitoneal fibrosis. The decision to offer chemotherapy must be done from case to case, taking into consideration performance status and organ dysfunction. Although corticosteroids are the most used drugs for idiopathic retroperitoneal fibrosis, there is no evidence of effectiveness when retroperitoneal fibrosis is secondary to malignancy. The only exception is retroperitoneal fibrosis related to carcinoid tumors, which can achieve great response to corticosteroids[14].

Despite the lack of effective systemic options for the management of retroperitoneal fibrosis associated with malignancy, these patients might draw benefit from palliative surgical approaches in order to relieve obstructive complications. Moreover, pain management is of great importance.

In summary, retroperitoneal fibrosis secondary to malignant disease is a rare condition associated with a dismal prognosis. Organ dysfunction and poor performance status usually preclude the use of systemic chemotherapy.

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Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23243641.

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Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.

Biology: *H. pylori*, *E coli*, *etc*.

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