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Colorectal cancer: a tale of two sides, or a continuum?

Mai Yamauchi[1], **Paul Lochhead**[1], **Teppei Morikawa**[1], **Curtis Huttenhower**[2], **Andrew T. Chan**[3,4], **Edward Giovannucci**[4,5], **Charles S. Fuchs**[1,4], and **Shuji Ogino**[1,6]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA

[2]Department of Biostatistics, Harvard School of Public Health, Boston, MA

[3]Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA

[4]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

[5]Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA

[6]Department of Pathology, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

Keywords

colon cancer; rectal cancer; subsite; tumor location; personalised medicine

INTRODUCTION

Colorectal cancer comprises a heterogeneous group of diseases that arise through varying molecular carcinogenic pathways, involving complex interactions between tumour cells and the host microenvironment.[1, 2] It has long been appreciated that developmental and physiological differences exist between anatomic segments of the colorectum, and that colorectal cancers occur with distinctly different frequencies at different subsites.[3] Whilst approaches to surgical and adjuvant therapy have set rectal cancer as a separate entity, colon cancers still tend get lumped together.

In the 1980's, epidemiological and molecular data suggested that cancers arising at different subsites may be biologically disparate, implying different cancer aetiologies or evolutions. These observations prompted the suggestion that the proximal and distal colon be considered separately in aetiological studies, with the splenic flexure as a demarcation point. [3] This dichotomisation has been propagated by subsequent clinical, translational, and epidemiological studies,[4–10] whilst evolving molecular data have galvanised and lent further support to the two-colon concept.[11–14] It remains uncertain, however, whether the splenic flexure represents a genuine or arbitrary divide in the aetiological spectrum of colon

Correspondence to: Shuji Ogino, MD, PhD, MS (Epidemiology) , Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Brigham and Women's Hospital, Harvard Medical School, 450 Brookline Ave., Room JF-215C, Boston, MA 02215 USA, Telephone: +1-617-632-1972; Fax: +1-617-582-8558, shuji_ogino@dfci.harvard.edu.
M.Y. and P.L. contributed equally.

cancers. Notably, our recent study,[15] demonstrating that the frequencies of key tumour molecular features change gradually along the length of the colon (rather than abruptly at the splenic flexure), challenges the current two-colon paradigm. The aim of this article is to review data relevant to the current concept of distinct molecular pathogeneses for proximal vs. distal colon cancers, describe the impact of our recent findings, and discuss the implications for future research.

THE EVOLUTION OF THE TWO-COLON CONCEPT

Historical Overture

There are incontrovertible differences between the ontogeny, morphology, biochemistry and physiology of the proximal and distal colon.[11–14] Furthermore, gross macroscopic differences are well described between proximal and distal colorectal tumours.[9, 11–14] In the early 1980's, epidemiological studies drew attention to sex and age disparities in site-specific colon cancer incidence, whilst contemporaneous reports highlighted an apparent upward trend in the incidence ratio of proximal to distal cancers.[3, 16, 17] Consequently, it was proposed that proximal and distal cancers may represent distinct disease entities.[3, 16, 18] The credibility of this hypothesis was bolstered by emerging cytogenetic and molecular data on the heterogeneity of colorectal cancer,[18, 19] including evidence that large scale chromosomal aberrations were relatively more frequent in distal cancers.[19]

An authoritative review of the evidence for genetically distinct proximal vs. distal colorectal cancer phenotypes was published by Bufill in 1990.[20] Numerous reports thereafter have supported the concept that proximal and distal cancers arise through distinct molecular pathways, and the two-colon concept has become the prevailing dogma.[11–14]

Genetic and Epigenetic Geography of Colorectal Cancer

In addition to clinical and pathological characteristics, cellular genomic and epigenomic determinants serve as important predictors of tumour evolution and progression.[21] Chromosomal instability (CIN), a common type of genomic instability in colorectal cancer, is characterised by widespread numeric chromosomal aberrations, subchromosomal amplifications, and loss of heterozygosity.[22] CIN is implicated in 60–70% of colorectal cancers, and is more commonly observed in distal compared to proximal cancers.[23, 24]

Microsatellite instability (MSI), characterised by somatic alterations in microsatellite repeat length, represents another distinct form of genomic instability observed in colorectal cancer. [25–27] In contrast to CIN, cancers that display a high degree of MSI (MSI-high) preferentially occur in the proximal colon.[23, 24, 28] MSI-high cancers constitute approximately 15% of all colorectal cancers and most frequently result from aberrant promoter hypermethylation and epigenetic silencing of the mismatch repair (MMR) gene *MLH1*.[23, 24, 28] *MLH1* promoter hypermethylation is most commonly a consequence of the CpG island methylator phenotype (CIMP-high), a form of epigenomic instability.[21, 29–32] MSI-high is also the hallmark of cancers arising in the context of the Lynch syndrome, where genetic predisposition is attributable to germline mutations in MMR genes. [23, 24, 28]

BRAF mutations are also more common in proximal colon cancers.[33–37] *BRAF* mutations are strongly associated with CIMP-high, which, in turn, correlates strongly with MSI-high.[33–37] Although CIMP-high and MSI-high cancers are both independently associated with proximal colonic location, only CIMP-high cancers are independently associated with *BRAF* mutation.[33–36]

An Epidemiological Scission

The apparent "left-to-right shift" in colorectal cancer incidence reported by epidemiological studies from the United States in the late 1970s and early 1980s,[16] lead to the adoption of the two-colon model by numerous incidence trend studies internationally.[38, 39] A true site-specific shift in incidence has not been a consistent finding of such studies. Nonetheless, age and sex-specific differences in proximal and distal cancer incidence continue to be described.[40]

Epidemiological studies, including molecular pathological epidemiology research,[1, 2, 41] have also employed the splenic flexure divide when investigating the effect of potential aetiological exposures. For example, prospective data from the Iowa Women's Health Study demonstrate that the relationship between smoking and colorectal cancer risk is strongest for proximal cancers, and for MSI-high, CIMP-high, and *BRAF*-mutated phenotypes.[42] The possible association between red or processed meat consumption and colorectal cancer risk is reportedly greater for distal cancers,[4] whilst the protective effect of regular aspirin use appears strongest for proximal cancers.[6]

Two Colons, Two Outcomes?

Data relating to colon cancer location and mortality are conflicting. Based on data from the German "Colon/Rectal Carcinoma" multicentre observational study, including 17,641 patients, it was reported that proximal colon cancers carry a significantly worse prognosis compared to distal cancers.[5] The size of this effect, however, diminished substantially following adjustment and stratification by disease stage. Furthermore, data from 53,801 colorectal cancer cases in the Surveillance, Epidemiology and End Results (SEER)-Medicare database, demonstrated no overall difference in five-year survival.[8] Interestingly, both of these studies showed a lower mortality for proximal vs. distal cancers for individuals with stage II disease. To date, tumour location has been largely neglected by oncology clinical trialists, and there is therefore a paucity of data on differences in outcome between proximal and distal colon cancers in relation to adjuvant therapy.[7]

A COLORECTAL CONTINUUM

Despite clear epidemiological, molecular, and clinicopathological correlates with colorectal cancer location, many individuals consider it implausible that these associations change abruptly at the splenic flexure. Nonetheless, the two-colon model predominates. It therefore remains uncertain whether the splenic flexure represents a genuine demarcation, or whether it is more a divide of convenience that polarises proximal-distal associations for markers that actually change gradually along the length of the colorectum. LaPointe and colleagues undertook an analysis of normal colonic tissue transcripts by microarray.[43] The gene expression data indicate the existence of two distinct groups of transcripts: a dominant group, whose expression pattern follows the classical dichotomous model, and a second group, where expression levels appear to change gradually along the proximal-distal axis of the bowel.[43] Worthley and colleagues observed a similar gradual transition along the normal colon for certain methylation markers, including *ESR1*, *HIC1*, and *APBA2*.[44]

Recent interest has focused on whether colorectal carcinogenesis might occur, through specific molecular mechanisms as a result of interactions between the gut microbiota, innate immune system, and other host factors, such as diet.[2, 45–48] Luminal contents, including gut microbial communities and their metabolites, might trigger initiating molecular events or, alternatively, influence the tumour microenvironment and promote neoplastic progression. Microbial and biochemical components of the luminal ecosystem, as well as interactions occurring within the mucosal-luminal interface, are likely to vary gradually along the proximal-distal axis of the colorectum.[49, 50] Indeed, this underlying continuum

was postulated, by LaPointe and colleagues, to be responsible for the apparent continuity observed in their gene expression data.[43]

Taking into account luminal biogeography, and the potential influence of host-microbial interactions, we recently investigated whether the frequencies of key colorectal cancer-related molecular characteristics also changed gradually through the multiple anatomic subsites.[15] Utilising a database of over 1400 colorectal cancers from two U.S. nationwide prospective cohort studies, we examined the frequencies of several molecular markers (CIMP, MSI, *BRAF*, *KRAS* and *PIK3CA* mutations, and LINE-1 methylation) in cancers arising throughout the colorectum.[15] We assessed the linearity and nonlinearity of molecular relationships by bowel subsite, with subsites defined as caecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, rectosigmoid junction, and rectum.[15] Notably, we found that the frequencies of CIMP-high, MSI-high, and *BRAF* mutation increased in a statistically linear fashion from the rectum to the ascending colon. Interestingly, caecal cancers appeared to represent a unique subtype, characterised by a high frequency of *KRAS* mutation. Our data demonstrate that the frequencies of molecular pathological changes in colorectal cancer transition gradually through bowel subsites, rather than altering abruptly at the splenic flexure.[15] Figure 1 illustrates the disparity between the prevailing two-colon concept and the continuum model. Importantly, our findings, and the continuum model, are not incompatible with previous observations. Our data do, however, support the necessity for a paradigm shift. We therefore propose a transition from the established dichotomous, or trichotomous (if one distinguishes rectum from distal colon), model towards a more detailed approach to the topography of colorectal carcinogenesis. Translation of the continuum model into a clinically practicable tool must be tempered by an appreciation of the limitations of lesion position reporting by clinicians, and by an awareness of inter-individual variability in colon length and mobility. Nonetheless, the adoption of a multisegmental model should be readily achievable.

FUTURE PERSPECTIVES

The potential limitations of the two-colon model for molecular biomarkers may extend to its application in other arenas. A recent examination of data from 39,568 participants in the German "Colon/Rectal Carcinoma" study suggests that certain clinicopathological features, such as histological subtype, show a seemingly linear correlation with anatomic subsite, whilst others, including disease stage, nodal status and lymphatic invasion, vary by subsite and do not fit neatly into a dichotomous model.[51] Indeed, our findings, suggesting molecular linearity from rectum to ascending colon and a potential unique molecular phenotype for caecal cancers, might have been masked by a two-colon study design.

We are at the dawn of the era of personalised colorectal cancer therapy and prevention.[52] As the oncological armament of chemotherapeutic and chemopreventive agents increases, careful disease phenotyping and subgroup identification is vital in ensuring optimal disease control whilst minimising toxicity.[52] For stage II disease, where site-specific survival differences appear to exist, adjuvant therapy remains controversial.[52] There is therefore clearly a need to define subgroups who might derive benefit from chemotherapy. Few colon cancer outcome studies have, however, considered the influence of tumor location and its potential interactions with molecular characteristics beyond the simple proximal-distal division.[5, 7, 53] In terms of screening, it is generally accepted that colonoscopy impacts less on mortality for proximal cancers compared to cancers arising in the distal colon.[13] If molecular strategies are to be employed in an attempt to increase the sensitivity of colonoscopy for proximal premalignant lesions, then a detailed appreciation of marker variation by subsite is essential.

Thus, a move towards the continuum model and multisegmental research design may prove informative not only for molecular and epidemiological investigators, but also for clinical outcome research. Furthermore, the ability to resolve variation in gut biogeography at subsite level seems imperative for "omics" research, and for a better understanding of effects of gut microbiome and host interactions on disease susceptibility and evolution.

In the first instance, multisegmental research design demands the collection of detailed colorectal subsite data for each neoplastic lesion in clinical, epidemiologic and pathological studies. One inevitable consequence of this study design will be the requirement for an increase in total sample size. This will be necessary in order to provide adequate statistical power necessary to permit meaningful subsite analyses. The larger sample size demanded by this model is therefore likely to promote pooling projects and multi-centre or multi-cohort collaborations in the near future.

CONCLUSION

The two-colon model has served us for over three decades.[11–14] Nonetheless, the emergence of data suggesting linearity in the frequencies of certain tumour molecular characteristics, and subsite-specific clinicopathological differences beyond the simple proximal-distal divide, support the need for a paradigm shift. In the era of personalised cancer therapy and prevention, future studies should consider adopting a multisegmental approach to bowel subsite in order both to advance our understanding of the complex aetiology of colorectal carcinogenesis, and to improve tailored preventative and therapeutic strategies.

Acknowledgments

Abbreviations

CIN	chromosomal instability
CIMP	CpG island methylator phenotype
MMR	mismatch repair
MSI	microsatellite instability
SEER	Surveillance, Epidemiology and End Results

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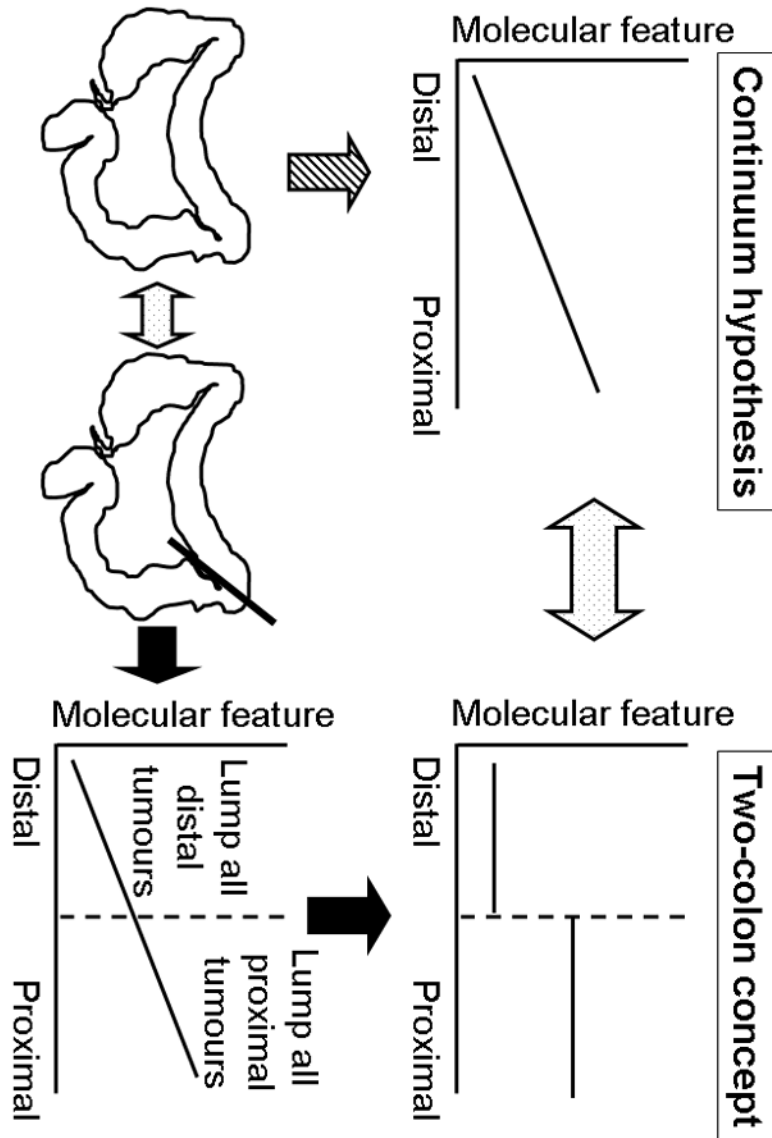
Figure 1.
Illustration of the disparity between the continuum model and the two-colon concept. The frequencies of molecular features such as CIMP-high, MSI-high, and *BRAF* mutation increase linearly from the rectum to the ascending colon according to the continuum model, and as supported by our recent data (left).[15] Previous studies have typically lumped together all distal tumours, and all proximal tumours, and compared the frequencies of various molecular features. Data from numerous previous two-colon studies appear to support the two-colon concept (right, top), even where the likelihood is that a continuum exists for the measured marker along proximal-distal axis of the bowel (right, bottom). The design of two-colon studies does not permit adequate evaluation of the linearity of marker frequency by colon subsite.