NEOPLASIA
www.neoplasia.com

Volume 20 Number 9 September 2018 pp. 943–950 943

Characterizing Microsatellite Instability and Chromosome Instability in Interval Colorectal Cancers

Amy L. Cisyk[*,†], Zoann Nugent[†], Robert H. Wightman[‡], Harminder Singh[†,§] and Kirk J. McManus[*,†]

[*]Department of Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, Canada; [†]Research Institute in Oncology & Hematology, CancerCare Manitoba, Winnipeg, Canada; [‡]Department of Pathology, University of Manitoba, Winnipeg, Canada; [§]Department of Internal Medicine, University of Manitoba, Winnipeg, Canada

Abstract

There are a substantial portion of colorectal cancers (CRCs), termed interval CRCs (I-CRCs), that are diagnosed shortly after a negative colonoscopy (i.e., no detectable polyps or CRC) and before recommended follow-up screening. The underlying cause(s) accounting for I-CRCs remain poorly understood, but may involve aberrant biology that drives genome instability. Genetic defects inducing genome instability are pathogenic events that lead to the development and progression of traditional sporadic (Sp-) CRCs. Classically, there are two genome instability pathways that give rise to virtually all Sp-CRCs, chromosome instability (CIN; ~85% of Sp-CRCs) and microsatellite instability (MSI; ~15% of Sp-CRCs); however, the contribution MSI and CIN have in I-CRCs is only beginning to emerge. To date, no study has simultaneously evaluated both MSI and CIN within an I-CRC cohort, and thus we sought to determine and compare the prevalence of MSI and/or CIN within population-based I-CRC and matched Sp-CRC cohorts. MSI status was established using a clinically validated, immunohistochemical approach that assessed the presence or absence of four proteins (MLH1, MSH2, MSH6 and PMS2) implicated in MSI. By combining the MSI results of the current study with those of our previous CIN study, we provide unprecedented insight into the prevalence of MSI and/or CIN between and within Sp- and I-CRCs. Our data show that MSI^+ tumors are 1.5-times more prevalent within I-CRCs than Sp-CRCs in a population-based setting and further show that CIN^+/MSI^+ I-CRCs occur at similar frequency as CIN^+/MSI^+ Sp-CRCs.

Neoplasia (2018) 20, 943–950

Introduction

CRC is the second leading cause of cancer-related deaths in North America. In 2017, ~162,250 North Americans were newly diagnosed with colorectal cancer (CRC), while an additional ~59,700 succumbed to the disease [2,3]. Further, of those newly diagnosed, ~80% to 85% are sporadic, or randomly occurring (i.e., there is no evidence for any predisposing hereditary cancer syndromes and/or family history) [4]. The high morbidity and mortality rates due to CRC underscore the need for accurate screening and diagnostic strategies [5].

Colonoscopy is an effective diagnostic and screening modality for CRCs and its use correlates with reduced CRC incidence and mortality primarily due to its ability to identify precursor lesions (i.e., polyps) and early stage disease (i.e., I and II) [6,7]. However, even with colonoscopies, there remain a portion of CRCs, termed interval CRCs (I-CRCs) that are diagnosed shortly after a negative colonoscopy (i.e., no detectable polyps or CRC) and before the recommended follow-up CRC screening. A meta-analysis determined that the pooled prevalence of I-CRCs is ~3.7% [8], which represents

Abbreviations: CIMP, CpG island methylator phenotype; CIN, Chromosome instability; CRC, Colorectal cancer; CS, CIN score; FISH, Fluorescent in situ hybridization; I-CRC, Interval CRC; IHC, Immunohistochemistry; MMR, DNA mismatch repair; MPE, Molecular pathologic epidemiology; MSI, Microsatellite instability; SD, Standard deviation; Sp-CRC, Sporadic CRC; TMA, Tumor micro-array.

Address all correspondence to: Research Institute in Oncology & Hematology, ON6010 – 675 McDermot Avenue, Winnipeg, MB, R3E 0V9. E-mail: Kirk.McManus@umanitoba.ca

Received 18 May 2018; Revised 31 July 2018; Accepted 31 July 2018

~6000 North Americans annually [2,3]. I-CRCs could represent missed sporadic CRC (Sp-CRC) cases that present as false-negative colonoscopies [9–15], or a unique subtype of Sp-CRC that harbors distinct biological characteristics [1,8,13,15–23] that enable rapid tumor growth and development (reviewed in [24]).

Genome instability is an enabling feature of virtually all cancer types [25] and is perhaps best understood in CRC. Genome instability typically arises through three pathways: 1) CpG island methylator phenotype (CIMP); 2) chromosome instability (CIN); and 3) microsatellite instability (MSI). While CIMP is an epigenetic phenomenon whereby hypermethylation of CpG islands on gene promoters correlates with gene silencing [26], CIN is defined as an increase in the rate at which chromosomes, or large chromosomal fragments, are gained or lost [27,28]. Finally, MSI arises from defects in the DNA mismatch repair (MMR) pathway [29]. Traditionally, MSI and CIN are proposed to be mutually exclusive pathways giving rise to Sp-CRCs [27], whereas it has been suggested that CIMP may underlie the development of MSI and/or CIN [30].

Currently, there is limited information detailing the etiological origins of I-CRCs. Of the few studies conducted, most have focused on the prevalence of MSI [18–20] and/or CIMP [17,19], with even fewer evaluating specific genes like *BRAF* [22], *KRAS* [21], or *CTNNB1* [16]. Collectively, these studies do support divergent biology relative to Sp-CRCs, as I-CRCs typically exhibit a 1.5- and 3.0-fold increase in the prevalence of CIMP and MSI, respectively. Given the traditional view that MSI and CIN are mutually exclusive [27,28], these observations imply that the prevalence of CIN is likely to be lower in I-CRCs (e.g. ~55%) versus Sp-CRCs (~80%-85%). However, we recently determined that the prevalence of CIN in I-CRCs (~85%) was statistically indistinguishable from Sp-CRC controls [1], raising the possibility that MSI and CIN may co-occur within I-CRCs.

No prior study has simultaneously assessed MSI and CIN within the same I-CRC cohort. We previously determined the prevalence of CIN within a tissue microarray (TMA) comprised of 95 Sp-CRC (control) and 46 I-CRC samples [1]. In the current study, we determined the prevalence of MSI within this same cohort and have now correlated these findings with the CIN findings of the previous study.

Materials and Methods

Ethics Statement

Ethics for this study, including the collection and use of archived clinical CRC tissue samples was approved by the University of Manitoba Research Ethics Board (REB Registry Number: H2010:237 [HS11032]) and Pathology Access Committee for Tissue and Manitoba's Health Information Privacy Committee.

Patient Identification

CRCs were identified from the population-based Manitoba Cancer Registry and linked to patient colonoscopy records through Manitoba Health databases as detailed elsewhere [1].

CRC Cohort

The CRC cohort is described elsewhere [1]. Briefly, I-CRCs were defined as CRCs diagnosed between 6 and 36 months following a colonoscopy, while CRCs detected upon initial colonoscopy (i.e. CRC diagnosis within a month of colonoscopy) were classified as Sp-CRCs and employed as controls. CRCs diagnosed between 1 to 6 months of colonoscopy were excluded from the analysis. The Sp-CRCs were matched 2:1 with I-CRCs based on gender, age and tumor location (proximal vs. distal based on location at or proximal to splenic flexure vs. more distally) [1]. Individuals with history of inflammatory bowel disease were excluded from both groups. Archived clinical formalin-fixed and paraffin-embedded tumor samples were supplied by the Department of Pathology in an anonymized, double-blinded fashion, with the I-CRC status only revealed following completion of the MSI analyses. A total of 141 samples, including 95 Sp- and 46 I-CRCs were evaluated. Minor sample attrition (5 Sp-CRCs and 1 I-CRC) occurred due to lack of informative CIN or MSI status stemming from too few cells for the CIN analyses, or lack of tumor tissue within the TMA cores based on routine hematoxylin and eosin staining, respectively.

CRC Tissue Micro-Array (TMA)

CRC samples were arrayed in duplicate as detailed previously [1].

Immunohistochemistry and Microsatellite Instability (MSI)

Immunohistochemistry (IHC) was performed using a Dako Autostainer Link 48 (Dako; Agilent) and clinically-validated monoclonal antibodies recognizing MLH1 (ES05 at 1:50; Dako), MSH2 (FE11 at 1:150; Dako), MSH6 (EP49 at 1:300; Dako) and PMS2 (EP51 at 1:50; Dako). Briefly, serial sections of the TMA (6 μm) were deparaffinized, subjected to an antigen retrieval and incubated with primary antibodies. Slides were mounted and scored in a double-blinded fashion for the presence (+) or absence (–) of each epitope interrogated. Samples lacking antibody labeling for 1 or more epitopes were considered MSI^+. To assess MSI within the CRC cohort, the presence (+) or absence (–) of four proteins (MLH1, MSH2, MSH6 and PMS2) with essential roles in MMR and causally linked with MSI were immunohistochemically assessed using clinically-validated antibodies. Briefly, serial sections of the CRC tissue microarray (TMA) were independently labeled with antibodies, and samples were qualitatively assessed for the presence or absence of each protein in a double-blinded manner (Figure 1). In agreement with standard clinical practice, samples exhibiting positive antibody labeling for each of the four proteins interrogated were defined as MSI^-, while samples lacking labeling for ≥1 targeted proteins were defined as MSI^+.

Chromosome Instability (CIN) Analysis

Briefly, the previous CIN study employed a FISH-based approach to assess gains and/or losses of three specific chromosomes implicated in CRC pathogenesis (i.e., chromosomes 8, 11, and 17) [1]. Chromosome enumeration probes recognizing each chromosome were quantitatively assessed within each CRC sample. To identify CIN^+ CRCs, we devised a metric, called a mean CIN Score (CS) that reflects both the gains and/or losses of each FISH probe (i.e., chromosome) within a given sample. A mean CS = 0 defines the diploid state and deviations from 0 identifies samples with gains and/or losses of FISH probes. As CIN and MSI typically occur in ~85% [27] and ~15% [29] of Sp-CRCs, respectively, we operationally defined the 15th percentile of the Sp-CRCs as the minimum threshold required to identify CIN^+ CRCs (i.e., mean CS ≥1.68) and determined that ~82% of I-CRCs (36/44 samples) were defined as CIN^+ tumors and were statistically indistinguishable from the 85% of Sp-CRCs (80/94 samples) defined as CIN^+ [1].

Statistical Analyses

Data were described using standard descriptive statistical analyses. Wilcoxon two sample tests were performed for continuous data such as comparing ages. Proportions were compared using Fisher's Exact