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Characterizing the Prevalence of Chromosome Instability in Interval Colorectal Cancer[1,2]

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Abstract

A substantial proportion of colorectal cancers (CRCs) are interval CRCs (I-CRCs; i.e., CRCs diagnosed soon after a colonoscopy). Chromosomal instability (CIN) is defined as an increase in the rate of which whole chromosomes/large chromosomal fragments are gained or lost and is observed in 85% of non-hereditary CRCs. The contribution of CIN to the etiology of I-CRCs remains unknown. We established a fluorescence *in situ* hybridization (FISH) approach to characterize CIN by enumerating specific chromosomes and determined the prevalence of numerical CIN in a population-based cohort of I-CRCs and control (sporadic) CRCs. Using the population-based Manitoba Health administrative databases and Manitoba Cancer Registry, we identified an age, sex, and colonic site of CRC matched cohort of I-CRCs and controls and retrieved their archived paraffin-embedded tumor samples. FISH chromosome enumeration probes specifically recognizing the pericentric regions of chromosomes 8, 11, and 17 were first used on cell lines and then CRC tissue microarrays to detect aneusomy, which was then used to calculate a CIN score (CS). The 15th percentile CS for control CRC was used to define CIN phenotype. Mean CSs were similar in the control CRCs and I-CRCs; 82% of I-CRCs exhibited a CIN phenotype, which was similar to that in the control CRCs. This study suggests that CIN is the most prevalent contributor to genomic instability in I-CRCs. Further studies should evaluate CIN and microsatellite instability (MSI) in the same cohort of I-CRCs to corroborate our findings and to further assess concomitant contribution of CIN and MSI to I-CRCs.

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Abbreviations: CRC, colorectal cancer; I-CRC, interval CRC; MSI, microsatellite instability; CIMP, CpG island methylator phenotype; CIN, chromosomal instability; FISH, fluorescence *in situ* hybridization; TMA, tissue microarray; CEP, chromosome enumeration probe; CS, CIN score; CS_8, CIN score for chromosome 8 within a nucleus or sample; CS_{11}, CIN score for chromosome 11 within a nucleus or sample; CS_{17}, CIN score for chromosome 17 within a nucleus or sample; CS_C, combined CIN score for chromosomes 8, 11, and 17 within a nucleus; $\overline{CS}$, mean CS for a given condition/population

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[2] This article refers to supplementary materials, which are designated by Supplementary Tables 1 to 5 and Supplementary Figures 1 to 3 and are available online at www.neoplasia.com.

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in North America, with more than 80% of these tumors arising randomly (i.e., without family history of affected first-degree relatives and/or predisposing hereditary syndromes), emphasizing the need for accurate screening and diagnostic strategies [1–3]. Colonoscopy is an accepted CRC screening test as it has been shown in observational studies to reduce CRC incidence and mortality by identifying CRCs at earlier stages as well as CRC precursor lesions (i.e., polyps) [2,4]. Furthermore, even when other approaches are used as the initial CRC screening test, colonoscopy is employed to investigate the positive results and diagnose CRC, and thus, most CRCs are now diagnosed on colonoscopy. However, even with colonoscopies, there remain a proportion of CRCs, termed interval CRCs (I-CRCs), that are diagnosed within a relatively short time period after a negative colonoscopy (i.e., colonoscopy that did not detect CRC). A recent meta-analysis estimated that approximately 1 in 27 CRCs is I-CRC [5], and if extrapolated to the statistics provided by the American Cancer Society, approximately 5200 Americans will be diagnosed with an I-CRC in 2014, and nearly 2000 will succumb to the disease [6]. Whether these tumors are missed sporadic CRCs that arise due to false-negative colonoscopies [7–13] or are a distinct subtype of CRC that harbor unique biologic properties [7,11,14–17] that result in rapidly developing tumors is currently unknown (reviewed in [18]).

Genome instability is a hallmark of virtually all tumor types and is arguably best described in CRC. In general, genome instability arises through one of three aberrant pathways: microsatellite instability (MSI), CpG island methylator phenotype (CIMP), or chromosomal instability (CIN) [1,19,20]. MSI arises due to defects in the DNA mismatch repair pathway [21] that result in subtle genomic alterations, while CIMP is an epigenetic phenomenon associated with DNA methylation and gene silencing [22]. CIN is defined as an increase in the rate at which whole chromosomes, or large parts thereof, are gained or lost, and thus, aneuploidy is often employed as a metric for CIN [23]. Within traditional sporadic CRC, MSI and CIN are generally accepted to be mutually exclusive pathways [23,24], while it has been proposed that CIMP may contribute to the development of MSI and/or CIN [25].

Currently, very little is known about the aberrant etiological origins of I-CRCs. Three studies have only examined the prevalence of the MSI [15,17] and CIMP [14,17] pathways within two distinct patient cohorts, and CIN has yet to be evaluated. Nevertheless, these studies demonstrated that I-CRCs do exhibit distinct biology relative to their traditional sporadic CRC counterparts. In fact, these studies revealed a 3.0- and 1.5-fold increase in the prevalence of MSI and CIMP, respectively, within I-CRCs relative to sporadic CRCs. Given the general observation that MSI and CIN are mutually exclusive, these results suggest that the prevalence of CIN within I-CRCs should be reduced. However, the prevalence of CIN is currently unknown in I-CRCs, and thus, it is critical to characterize its potential contribution to the pathogenesis of these tumors.

In this study, we established and employed a fluorescence *in situ* hybridization (FISH)–based approach to evaluate numerical CIN within a Manitoban cohort of I-CRCs and sporadic CRCs. Aneusomy (i.e., abnormal chromosome numbers) was used as the metric for CIN, and through chromosome enumeration within patient-derived tumor samples, we identified the extent of CIN within I-CRCs to be nearly identical to that of the matched control/sporadic CRCs. More specifically, the chromosome enumeration results showed no statistically significant differences between the interval and sporadic cohorts for each subcategory (i.e., gender, age, tumor location, and so on). Our data show that CIN is frequently observed in I-CRCs and further suggest that it likely contributes to the development of these tumors. Finally, due to the similar CIN profiles observed in both I-CRCs and sporadic CRCs, our findings suggest that missed sporadic CRCs may be a predominant factor in the development of I-CRCs.

Materials and Methods

Ethics Statement

This study, including the collection and use of archived clinical CRC tissue samples, was approved by the University of Manitoba Research Ethics Board and Pathology Access Committee for Tissue and Manitoba's Health Information Privacy Committee.

Cell Culture

HeLa cells were purchased from American Type Culture Collection (Manassas, VA) and are a hypotetraploid cervical adenocarcinoma cell line with a modal number of 82 chromosomes, while hTERT cells are a diploid, immortalized fibroblast cell line with a modal number of 46 chromosomes [26] that were generously provided by Dr C. P. Case (Bristol University, Bristol, United Kingdom). Cells were grown in Dulbecco's modified Eagle's medium (HyClone) supplemented with 10% FBS at 37°C in a humidified incubator with 5% CO_2.

Patient Identification

Manitoba Health is the publicly funded health insurance agency that provides health care coverage to all Manitoba residents. Manitoba Health maintains a number of electronic databases, including hospital discharge and physician claims, for monitoring and accounting purposes [27]. Every resident of Manitoba is assigned a unique personal health identification number, which can be used to link patient records longitudinally. For the current study, CRCs occurring in Winnipeg residents (the largest provincial city with two thirds of the Manitoba residents) were identified from the population-based Manitoba Cancer Registry (which tracks all cancers diagnosed in the province) and linked to patient colonoscopy records through Manitoba Health databases to identify I-CRCs and control CRCs. Medical records of colonoscopies were reviewed to determine the differences in colonoscopies in the two groups.

CRC Cohort

For the purpose of this study, I-CRCs were defined as CRCs diagnosed between 6 and 36 months following a colonoscopy, while CRCs detected on initial colonoscopy (on the date of the colonoscopy or within a month thereafter) were classified as sporadic and were included as controls. Sporadic CRCs were matched 2:1 to I-CRC by age (± 5 years), gender, and tumor location in the colon (i.e., right *vs* left). CRCs occurring in and proximal to the splenic flexure were considered right-sided CRC and those more distally left-sided CRC. Only CRCs diagnosed between 1 January 2007 and 30 March 2010 were included. Exclusion criteria included patients with prior CRC or inflammatory bowel disease, as well as patients diagnosed with CRC before the age of 50 years, due to the higher probability of a hereditary predisposition for CRC.