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Original contribution

Are clinicopathological features of colorectal cancers with methylation in half of CpG island methylator phenotype panel markers different from those of CpG island methylator phenotype–high colorectal cancers?☆,☆☆

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Summary CpG island methylator phenotype (CIMP)–high (CIMP-H) colorectal cancer (CRC) is defined when a tumor shows methylation at greater than or equal to 60% of CIMP panel markers. Although CRCs with methylation at 50% of panel markers are classified as CIMP-low/CIMP-0 tumors, little is known regarding the clinicopathological and molecular features of CRCs with methylation at 4/8 panel markers (4/8 methylated markers) and whether they are akin to CIMP-H or CIMP-low/CIMP-0 CRCs in terms of their clinicopathological or molecular features. A total of 1164 cases of surgically resected CRC were analyzed for their methylation status in 8 CIMP panel markers, and the frequencies of various clinicopathological and molecular features were compared between CRCs with 0/8, 1/8 to 3/8, 4/8, and 5/8 to 8/8 methylated markers. CRCs with 4/8 methylated markers were closer to CRCs with 5/8 to 8/8 methylated markers in terms of sex distribution, mucin production, serration, nodal metastasis, CK7 expression, CK20 loss, and CDX2 loss frequencies and overall survival rate. CRCs with methylation at 4/8 markers were closer to CRCs with 1/8 to 3/8 methylated markers in terms of less frequent right colon location and poor differentiation. CRCs with 4/8 methylated markers showed the shortest overall survival time compared with CRCs with 0/8, 1/8 to 3/8, 4/8, or 5/8 to 8/8 methylated markers. In terms of clinicopathological

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and molecular features, CRCs with 4/8 methylated markers appeared to be closer to CIMP-H than to CIMP-low/CIMP-0 and would thus be better classified as CIMP-H if the CRCs require classification into either CIMP-H or CIMP-low/CIMP-0.

1. Introduction

Colorectal cancer (CRC) encompasses a multistep progression from benign tumor into malignancy. The classical pathway, so-called adenoma-carcinoma sequence, is a concept that refers to the development of adenocarcinomas from preexisting conventional adenomas. Chromosomal instability acts as the driving force for the classical pathway. Vogelgram depicts genetic alterations occurring in the progression of chromosomally unstable colorectal tumors. However, conventional adenomas are unlikely to be premalignant lesions for sporadic microsatellite instability (MSI)–high (MSI-H) or CpG island methylator phenotype (CIMP)–high (CIMP-H) CRCs because CIMP-H tumors are rarely, if ever, found in conventional adenomas [1,2]. The serrated neoplasia pathway has been proposed to refer to a progression of serrated lesions, including sessile serrated adenomas (SSAs)/polyps or traditional serrated adenomas, to colorectal adenocarcinomas [3]. CIMP-H or sporadic MSI-H CRCs are thought to develop through the serrated neoplasia pathway.

CIMP-H CRCs are known to have characteristic clinicopathological and molecular features, such as predominance in females and high frequencies of *BRAF* mutation, MSI-H, right colon location, mucinous histology, and poor differentiation [2,4-6]. In contrast, CIMP-low CRCs (CRCs with a lesser degree of CIMP-specific methylation) are indistinct in their clinicopathological and molecular features [7], which might be attributed to the absence of marker panels available to specifically detect CIMP-low CRCs or a heterogeneous composition of CIMP-low CRCs by both carcinomas from the classical pathway and carcinomas from the serrated neoplasia pathway. Although traditional serrated adenomas are generally thought to be the premalignant lesions of CIMP-low CRCs [8], most CIMP-low CRCs are likely to arise in conventional adenomas. This has been shown in the following 2 studies: (1) in our previous study analyzing methylation status of 8 CIMP panel markers in carcinoma ex-adenoma samples, CIMP-low was found in 41% of carcinoma samples that were contiguous with tubular or tubulovillous adenomas [9], and (2) a recent genome-wide methylation study demonstrated similar DNA methylation signatures between a subset of tubular adenomas and CIMP-low CRCs [10]. However, a considerable proportion of SSAs with dysplasia are not CIMP-H [11], raising the possibility that adenocarcinomas arising from SSAs might not be always CIMP-H or that the present cutoff value for the diagnosis of CIMP-H might be so high that a considerable portion of SSAs are not diagnosed as CIMP-H.

Using the 8-marker panel, CIMP-H is defined as greater than or equal to 5/8 or 6/8 methylated markers. Although CIMP-low CRCs are heterogeneously populated by both carcinomas from the classical pathway and carcinomas from the serrated neoplasia pathway [8,12], CRCs with 4/8 methylated markers are unlikely to have progressed from the classical pathway. In our previous study of carcinoma ex-conventional adenoma samples, the average number of methylated panel markers was 0.9 (SD, 1.15) and 0.7 (SD, 0.95) in the adenoma and carcinoma components, respectively, indicating that most of the classical pathway carcinomas (97.5%) contain less than 4/8 methylated markers. Thus, CRCs with 4/8 methylated markers are likely to have progressed from the serrated neoplasia pathway. In the present study, we aimed to characterize CRCs with 4/8 methylated markers for their clinicopathological and molecular features by comparing these with CIMP-low ($\leq$3/8 methylated markers) and CIMP-H ($\geq$5/8 methylated markers). We analyzed 1164 cases of surgically resected CRC to obtain methylation status in 8 CIMP panel markers and assessed the frequencies of various clinicopathological and molecular features in relation to the number of methylated markers (from 0 to 8).

2. Materials and methods

2.1. Patients

This study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea. We reviewed tissue slides of primary CRC cases that were surgically resected from 2004 to 2007 in Seoul National University Hospital. Exclusion criteria included (1) noninvasive cases, (2) familial adenomatous polyposis, (3) cases with neoadjuvant chemotherapy and/or radiotherapy, (4) multiple occurrences, and (5) recurrent tumors. Clinicopathological features, including age, sex, tumor location, and TNM staging (American Joint Committee on Cancer Sixth Edition) [13], were obtained from the electronic medical record. Through microscopic examination of representative sections of tumors, 2 pathologists (Bae and Kang) evaluated the following parameters: tumor differentiation (tumor grade), Crohn-like lymphoid reaction, luminal serration, and extraglandular mucin production [6]. After selecting the tissue slides that represented main histology and differentiation, areas in which the tumor cells were the most dense were marked under