Integrated Analysis of the Endoscopic, Pathological and Molecular Characteristics of Colorectal Tumorigenesis

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Keywords

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Abstract

Background: Colorectal cancers (CRCs) develop through the accumulation of genetic and epigenetic alterations of oncogenes and tumor suppressor genes. In addition to the well-characterized adenoma-carcinoma sequence, the serrated neoplasia pathway is now recognized as an alternative pathway for CRC development. **Summary:** Through analysis of the colonoscopic, pathological, and molecular features of colorectal tumors, we identified a novel microsurface structure characteristic of serrated lesions. The Type II-Open (Type II-O) pit pattern is highly specific to sessile serrated adenoma/polyps (SSA/Ps), and Type-II-O-positive tumors frequently exhibit v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation and 5'-C-phosphate-G-3' (CpG) island hypermethylation. By screening DNA methylation associated with the development of serrated lesions, we detected methylation of secreted protein acidic and rich in cysteine (SPARC)-related modular calcium binding 1 (SMOC1) in tra-

ditional serrated adenomas (TSAs). Epigenetic silencing of SMOC1 is prevalent among TSAs but it is rarely observed in SSA/Ps, which suggests SMOC1 could be a useful diagnostic marker of serrated lesions. We also searched for epigenetic alterations associated with the growth pattern of colorectal tumors and found that methylation of neurotensin receptor 1 is associated with lateral and non-invasive tumor growth.

Key Message: Through the summarized studies, we have been able to identify novel morphological and molecular features that could contribute to a better understanding of colorectal tumors and to improved clinical diagnosis.

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide. CRCs develop through the accumulation of multiple genetic and epigenetic alterations of oncogenes and tumor suppressor genes. In the well-characterized adenoma-carcinoma sequence model, CRCs arise from adenomas through the accumulation of genetic alterations of key genes that include APC, v-Ki-ras2 kirsten rat sarcoma viral oncogene homolog (KRAS), and tumor protein p53 (TP53) (Fig. 1) [1]. In

addition, advances in cancer genome biology revealed that CRCs can be categorized into several subgroups based on their molecular characteristics. For instance, CRCs are broadly divided into 2 groups based on whether they exhibit chromosomal instability or microsatellite instability (MSI) [2]. From an epigenetic viewpoint, moreover, a subset of CRC exhibit concurrent hypermethylation of multiple CpG islands, which is referred to as the 5'-C-phosphate-G-3' (CpG) island methylator phenotype (CIMP) [3]. CRCs with CIMP exhibit several characteristic features, including frequent v-ras murine sarcoma viral oncogene homolog B1 (BRAF) mutation, infrequent TP53 mutation, loss of mutL homolog 1 (MLH1) expression due to CpG island hypermethylation, and resultant MSI [3].

In addition to the adenoma-carcinoma sequence, the serrated neoplasia pathway is now recognized as an alternative pathway toward CRC development [4]. Serrated lesions are subcategorized into hyperplastic polyps (HPs), sessile serrated adenoma/polyps (SSA/Ps), and traditional serrated adenomas (TSAs) [4]. In the past, serrated lesions were simply classified as HPs and considered to be without malignant potential. However, evidence now strongly indicates that SSA/Ps and TSAs are important premalignant lesions. In particular, because SSA/Ps frequently exhibit BRAF mutation and hypermethylation of multiple CpG islands, SSA/Ps are thought to be precursors of CRCs with MSI (Fig. 1) [4]. To understand the molecular basis of colorectal tumorigenesis and apply that knowledge to achieve better diagnosis and treatment of CRC, we carried out an integrative analysis of the colonoscopic, pathological and molecular characteristics of premalignant and malignant colorectal lesions. In this review, we describe correlations between the morphological features and molecular alterations in colorectal lesions as well as novel epigenetic events potentially associated with tumor development.

Associations between Microsurface Structures and Molecular Alterations in Serrated Lesions

High-resolution magnifying colonoscopy is a powerful diagnostic tool for evaluating the malignant potential of colorectal lesions [5, 6]. Using Kudo's classification, the pit patterns of non-neoplastic lesions are classified as Type I (normal) or Type II (HP), while the pit patterns of neoplastic lesions are classified as Types III, IV, and V, among which Type V lesions are considered to be cancers [5]. When this pit pattern classification was first estab-

Fig. 1. Models of CRC development. Shown are the adenoma-carcinoma sequence and the serrated neoplasia pathway.

Fig. 2. Microsurface structures (pit patterns) of hyperplastic polyps and SSA/Ps. The Type II-O pit pattern is a characteristic feature of SSA/Ps, and Type II-O-positive tumors frequently show BRAF mutation and hypermethylation of multiple genes, including p16 and IGFBP7.

lished, SSA/Ps and TSAs were considered to be non-neoplastic lesions because they exhibit the Type II pit pattern. However, we hypothesized that serrated lesions with neoplastic potential may present specific morphological features that are distinct from non-neoplastic lesions. By comparing the morphological features of a series of serrated lesions and conventional adenomas, we found that SSA/Ps possess a characteristic pit pattern that is similar to the conventional Type II, but the pits were wider and more rounded in shape, reflecting crypt dilation [7]. We termed this pattern Type II-Open (Type II-O) and further analyzed its clinical and biological significance (Fig. 2).

The Type II-O pattern was highly specific to SSA/Ps, and a majority of Type II-O-positive lesions exhibited BRAF mutation and CIMP, while they infrequently showed KRAS mutation [7]. These results suggest that the Type II-O pit pattern may be a hallmark of lesions with the potential to develop into CIMP-positive/MSI CRCs.

To test this idea, we analyzed lesions in which portions exhibiting Type II-O patterns were present along with portions exhibiting more advanced pit patterns (Type III, IV or V). Both subcomponents exhibited similar molecular features (BRAF mutation and CIMP), suggesting they arose from a single origin, though subcomponents with advanced pit patterns showed higher levels of p16 and MLH1 methylation.

In a subsequent study, we further investigated the clinical implications of the Type II-O pit pattern. SSA/Ps with cytological dysplasia are reportedly characterized by frequent MLH1 methylation and are at a high risk of developing into CRC [8]. We found that the Type II-O plus Type III/IV pit pattern is a common feature of SSA/Ps with cytological dysplasia in both the proximal and distal colon [9], which suggests that progression of SSA/Ps to more advanced lesions is associated with further accumulation of molecular alterations and morphological changes.

We also explored the microsurface structures characteristic of TSAs and recently reported that Type II pit patterns could be subcategorized into classical Type II, Type II-O, and Type II-Long (Type II-L) [10]. Most lesions with simple Type II or Type II-L patterns were HPs, while mixtures of Type II or Type II-L plus Type III/IV patterns were characteristic of TSAs. We therefore propose that Type II-L-positive TSAs may develop into KRAS-mutated/CIMP-low/microsatellite stable CRCs, although further study will be necessary to confirm this hypothesis.

Epigenetic Silencing of Secreted Protein Acidic and Rich in Cysteine-Related Modular Calcium Binding 1 in TSAs

In contrast to SSA/Ps, TSAs are thought to develop into microsatellite stable CRCs, though the underlying mechanism of tumor progression is not yet fully understood [11]. TSAs reportedly exhibit genetic and epigenetic characteristics distinct from those of SSA/Ps. For instance, PTPRK-RSPO3 fusion and RNF43 mutations are reported to be characteristic features of TSAs [12, 13]. TSAs mostly harbor KRAS or BRAF mutations, and those with BRAF mutations have features similar to SSA/Ps, such as a proximal colon location and CIMP-high [11, 14]. However, TSAs rarely show MLH1 methylation. CIMP-high is infrequently found in KRAS mutant and BRAF/KRAS wild-type TSAs, while approximately half of these lesions show CIMP-low [11]. These results indicate that aberrant DNA methylation likely plays an important role in the development of TSAs.

To identify DNA methylation associated with the TSA development, we analyzed a series of TSAs containing both protruding and flat components within the same tumors [15] (Fig. 3). As the flat components are considered precursors of the protruding portions, we compared the genome wide DNA methylation status between the 2 components. We identified 11 genes (B3GALNT1, CADPS, FAM92A1, FEZ1, FRMD4B, GABRA4, KIAA1529, OGFRL1, PRDM16, SMOC1, and ZNF34) in which methylation levels were elevated in the protruding components. Among them, we noted that secreted protein acidic and rich in cysteine (SPARC)-related modular calcium binding 1 (SMOC1) was frequently methylated in TSAs but was rarely methylated in SSA/Ps. SMOC1 belongs to the SPARC family of matricellular proteins, which has 8 known members: SPARC, SPARCL1/Hevin, SPOCK1, -2, -3, SMOC1, -2 and FSTL1. Although the biological function of SMOC1 is not fully understood, it is reportedly associated with osteoblast differentiation, ocular and limb development, and angiogenesis [15–17]. Members of SPARC family have been implicated in various tumor types. For instance, SPARC is silenced by DNA methylation in CRC and it may act as a tumor suppressor [18, 19]. SMOC1 is known to be overexpressed in brain tumors and methylated in breast cancer, but its role in tumorigenesis remains largely unknown [20, 21].

Our immunohistochemical analysis revealed that SMOC1 expression is reduced in TSAs, whereas it is abundantly expressed in normal colon and SSA/Ps [15]. This suggests that SMOC1 could be a useful marker for discriminating SSA/Ps from TSAs. Moreover, lesions in which TSA and cancerous components were present together showed higher levels of SMOC1 methylation than TSAs without cancer, indicating that SMOC1 methylation may be associated with the risk of CRC development.

When we further analyzed SMOC1 methylation in a large number of clinical specimens, we found that SMOC1 was also frequently methylated in conventional adenomas and CRCs [15]. For non-invasive tumors, SMOC1 methylation was positively associated with older age, larger tumor size, KRAS mutation, TP53 mutation, and CIMP-low. In invasive tumors, SMOC1 methylation was again associated with KRAS mutation and CIMP-low. Analysis using CRC cell lines showed that SMOC1 methylation is associated with gene silencing, and ectopic expression of SMOC1 suppressed CRC cell proliferation and xenograft formation in nude mice, which is indicative of its tumor suppressor function. Collectively, these results suggest that SMOC1 methylation may play a key role in the development of TSAs and adenomas that could progress to CIMP-low CRCs [15].

Fig. 3. Identification of SMOC1 methylation in TSA. **a** To identify molecular alterations associated with TSA development, biopsy specimens were collected from flat and protruding components of TSAs, after which the molecular alterations were analyzed. **b** DNA methylation profiles were compared between the flat and protruding components of TSAs. CpG sites differentially methylated between the 2 components were selected, and SMOC1 methylation was identified.

Fig. 4. A screen for genes associated with the growth patterns of colorectal tumors. **a** By comparing DNA methylation profiles between large/non-invasive and small/invasive tumors, frequent NTSR1 methylation in large/non-invasive tumors was identified. **b** NTS-NTSR1 signaling promotes tumor cell survival, proliferation, migration, and invasion.

Epigenetic Silencing of Neurotensin Receptor 1 is Associated with Lateral and Non-Invasive Tumor Growth

In contrast to the classical adenoma-carcinoma sequence, a subset of CRCs develop from non-polypoid lesions. Laterally spreading tumors (LSTs) are tumors that extend laterally along the luminal wall and are greater than 10 mm in diameter with a low vertical axis [22]. LSTs are categorized into 2 subtypes: those with a granular morphology (LST-G) and those with a flat or non-granular morphology (LST-NG) [23, 24]. Although the molecular mechanism involved in the development of LSTs is not fully understood, several studies reported frequent detection of KRAS mutation and CIMP in LSTs [25, 26]. A more recent study showed that LST-G is associated with KRAS mutation and the intermediate-methylation epigenotype, while LST-NG is characterized by catenin beta-1 (CTNNB1) mutation and a low-methylation epigenotype [27].

The above findings suggest that aberrant DNA methylation may be an important determinant of the lateral or

vertical growth pattern of colorectal tumors. We therefore compared the DNA methylation profiles of large (≥ 20 mm in diameter) and non-invasive tumors with those in small (< 20 mm in diameter) and invasive tumors (Fig. 4) [28]. We found that a substantial number of genes were differentially methylated among these tumor types and identified neurotensin receptor 1 (NTSR1) as a gene that is preferentially methylated in large, non-invasive tumors. Neurotensin (NTS) is a 13-amino acid neuropeptide localized mainly in the central nervous system and the distal small bowel [29]. The physiological functions of NTS include modulation of gastrointestinal tract motility, stimulation of intestinal secretion, and promotion of the growth and regeneration of intestinal epithelial cells [29]. Activation of NTS-NTSR1 signaling has been implicated in the progression of various cancers, including CRC [30].

Among non-invasive colorectal tumors, levels of NTSR1 methylation were significantly higher in LST-G than other types of tumors [28]. We also noted that higher levels of NTSR1 methylation were associated with bet-

ter overall survival of patients with invasive colorectal tumors. Analysis using CRC cell lines showed that NTSR1 methylation is associated with its transcriptional silencing, and that ectopic expression of NTSR1 promoted CRC cell proliferation and invasion, which is consistent with the known oncogenic function of NTS-NTSR1 signaling (Fig. 4) [30].

The results summarized above suggest that despite functioning as an oncogene, NTSR1 is a target of epigenetic silencing in colorectal tumors. To investigate the biological significance of NTSR1 methylation in colorectal tumorigenesis, we examined mixed colorectal lesions possessing adenomatous and malignant components. We found that NTSR1 was methylated and silenced in the adenomatous regions but was unmethylated and abundantly expressed in cancerous regions [28]. This suggests that NTSR1 is a methylation-prone gene early during tumorigenesis, but its activation is necessary for malignant progression. It remains unclear, however, whether tumor cells actively demethylate NTSR1 or whether there is selective survival of tumor cells without NTSR1 methylation during malignant progression. These results suggest that NTSR1 methylation may be associated with the lateral and non-invasive growth of colorectal tumors and could be a prognostic marker of CRC.

Conclusions

In a series of studies, we have shown that microsurface structures are associated with molecular alterations and progression of serrate lesions. As colonoscopic identification and resection of lesions with malignant potential is an effective strategy for CRC prevention, our findings could contribute to better identification and treatment of premalignant colorectal lesions. In addition, through performance of an integrated analysis of endoscopic, pathological, and molecular characteristics, we identified novel epigenetic alterations potentially associated with the development of colorectal tumors. Although further study will be necessary to fully understand the roles of these genes in tumorigenesis, they appear to have the potential to serve as diagnostic or predictive markers of colorectal tumors.

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Disclosure Statement

All authors have no conflict of interest to declare.

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