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Review

Targeting the serrated pathway of colorectal cancer with mutation in *BRAF*

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ABSTRACT

A recently acknowledged morphological pathway to colorectal cancer originates from precursor polyps with a serrated appearance due to branching and folding of the colon epithelium. This serrated origin accounts for up to 30% of all colorectal tumors but these are heterogeneous regarding molecular characteristics and patient outcome. Here we review the current knowledge about the classification of this tumor subtype and its association with five key features: mutation status of the *BRAF* or *KRAS* genes, the CpG island methylation phenotype, microsatellite instability, immune cell infiltration, and overexpression of GTPase RAC1b. Subsequently, available therapeutic approaches for targeting these molecular characteristics are presented and critically discussed.

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1. Introduction

Cancer of the colon and rectum (CRC) is one of the most prevalent tumors worldwide, especially in the economically developed regions [1]. According to the Globocan 2012 data collected by the International Agency for Research on Cancer for both sexes, CRC presented with over 1.35 million cases the third most common incidence (following lung and breast cancers) and the fourth cause of cancer mortality worldwide [2]. Risk factors have been identified in epidemiological studies and include family histories of either colorectal cancer or inflammatory bowel disease, but the disease burden at the population-level is mainly accounted for by modifiable factors such as smoking, excessive alcohol consumption, high consumption of red and processed meat, obesity, and diabetes [1].

The development of sporadic CRCs has been extensively studied and reviewed [1,3–7]. The majority of the sporadic tumors derives from premalignant precursor lesions known as dysplastic tubular or villous adenomas (also called adenomatous polyps) and develops through an adenoma–carcinoma sequence. They further exhibit widespread chromosomal abnormalities, designated as the chromosomal instability phenotype (CIN). By contrast, they retain functional DNA mismatch repair so that repetitive nucleotide sequence stretches, such as the microsatellite markers, remain unchanged (microsatellite stability phenotype, or MSS). Typical somatic mutations are found in the *APC* and *TP53* tumor suppressor genes, or in the oncogene *KRAS* [1,3–7].

A second major group of sporadic CRC includes about 15% of patients and is characterized by different molecular events. These tumors preferentially occur in the proximal colon, have a stable chromosome number, but show a high rate of DNA sequence mutations (microsatellite instability phenotype, or MSI) caused by deficient DNA mismatch repair [3]. The majority of these tumors further presents a serrated polyp morphology [8,9] and derives from the group of hyperplastic polyps, as described in more detail below.

Finally, hereditary forms contribute an additional 3–5% to all CRC cases.

2. The serrated pathway to colorectal cancer

2.1. Molecular and morphological characteristics

Serrated polyps display a typical morphology with a serrated folding of the crypt epithelium that likely result from increased epithelial mass due to a combination of hyperproliferation in the crypt and reduced apoptosis of differentiated cells [10]. They can form in all sections of the colon and were first described about 25 years ago [11–13].

However, serrated carcinomas, which contribute to roughly 30% of all CRC cases, evolve mostly from precursor serrated polyps that are localized in the proximal colon. Notably, serrated polyps form a heterogeneous group with certain histological characteristics associated with a preferential colonic localization. They are usually classified as either hyperplastic polyps (HPs), sessile serrated adenoma (SSA), or traditional serrated adenoma (TSA) [9,14–19]. HPs are the most frequent serrated polyp (75%) and represent around 30% of all colonoscopically detected colon polyps. They exhibit an elongated symmetric architecture and are mostly found in the distal colon, being generally benign when smaller than 5 mm. By contrast, the subtype of microvesicular HP (MVHP), occurs in the proximal colon, shares morphologic and molecular features with serrated adenomas and can progress to malignant serrated carcinomas [9]. SSAs grow as broad-based flat polyps on the mucosa surface of the proximal colon, represent up to 9% of all colon polyps and account for up to 25% of the serrated polyps. They frequently display crypt branching and dilatation at the crypt base. SSAs represent the main precursor lesion for about half the serrated carcinomas (about 12% of all sporadic CRC tumors) and are characterized by a high level of MSI (MSI-H). Finally, TSAs appear usually in the distal colon and are rare (2–5% of all colorectal polyps) with distorted tubulovillous (filiform) architecture, resembling in part the 'traditional' adenomatous polyps, and show the presence of ectopic crypt foci [20,21]. In addition, the admixed occurrence of TSA adjacent to HP, SSA, or conventional adenomas is frequent (up to 52% of TSAs), so that morphological classification is sometimes difficult to score and contributes to different frequency values for serrated polyps described in the literature [22]. Table 2 summarizes the main features that distinguish the different serrated polyp types (for histo-pathological details see the specialized reviews [7,14,16,20,23]).

Three molecular features that distinguish the serrated pathway deserve more detailed consideration: mitogen-activated protein kinase (MAPK) pathway activation, CpG island methylator phenotype (CIMP), and microsatellite instability.

Two early tumor-initiating events are mutations in either the *KRAS* or *BRAF* genes that both stimulate proliferation downstream of the epidermal growth factor receptor (EGFR) and along the same MAPK pathway. These mutations have already been detected when microdissected premalignant polyps or aberrant crypt foci were genotyped and revealed to be alternative events [24–31]. Mutation detection is important as it identifies tumors resistant to anti-EGFR therapy, either through receptor-blocking monoclonal antibodies or inhibitors of the receptor tyrosine kinase activity. Indeed, in clinical practice only around 10% of cases respond to anti-EGFR therapy [32,33].

A highly significant association exists between the presence of mutation in *BRAF* and genome-wide DNA hypermethylation at or around the CpG islands present in the majority of human gene promoters [34–36], This tumor phenotype is called CpG island methylator phenotype (CIMP) and results in epigenetic silencing of gene expression at the methylated promoters, for example of the genes encoding cell cycle inhibitor p16INK4a, the TP53 regulator p14ARF, and the DNA repair factor MGMT [37]. The inhibition of apoptosis that is observed in serrated adenomas has been proposed to be a functional consequence of CIMP. A genome-wide study indicated subsequently that CpG island-methylation was directly responsible for the silencing of at least 112 genes [34] but also that a large number of "passenger" DNA hypermethylation events exist in the promoters of non-expressed genes. Thus, CIMP represents a broad genome-wide change in epigenetic regulation.

Two distinct CIMP phenotypes have been recognized in the serrated pathway to CRC [34,38]: CIMP-high (CIMP-H) was defined by methylation in the majority of five selected CIMP marker gene promoters (see [39] for a history of CIMP panels used) and is a characteristic feature of sporadic MSI-H tumors with *BRAF* mutation. These tumors occur preferentially in the proximal colon, are responsible for 10–15% of all CRC cases and more prevalent in female patients. They derive from SSAs or sometimes MVHP [9,23,31] and have a better prognosis than other CRC subtypes, which is attributed to the observed high infiltration of immune cells. The serrated CIMP-H/*BRAF* CRC group is further characterized by a statistically significant association between CRC and smoking [40].

By contrast, a CIMP-low (CIMP-L) phenotype shows methylation in only few of the above CIMP markers and is associated with *KRAS* mutation and MSS or low level MSI (MSI-L) status. Such tumors are mostly derived from TSAs with more adenomatous features and occur in the distal colon with a poor prognosis [22]. These characteristics can be replicated upon expression of mutant *KRAS* in the intestinal epithelium of transgenic mice [41]. A recent genome-wide DNA methylation profiling has confirmed these two CIMP classifications and suggested the use of specific marker genes to distinguish between CIMP-H and -L [34]. An additional, related subgroup of TSA with more serrated features is characterized by *BRAF* mutation and CIMP-H but retains mismatch repair function with a MSS or low level MSI (MSI-L) status. In both these TSA groups, malignant progression involves TP53 mutation and WNT pathway activation [21, 23,42], conferring poor prognosis [43]. Wnt pathway activation in TSA has been evidenced by a shift to nuclear β-catenin staining; however, typical loss-of-function mutations of the *APC* gene are