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Genetics of the Serrated Pathway to Colorectal Cancer

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Accounting for ~15% of all colorectal cancers (CRCs), the serrated pathway represents an alternate mechanism of colorectal carcinogenesis that yields microsatellite stable (MSS) tumors and the overwhelming fraction of "sporadic" microsatellite instability high (MSI-H) tumors. Moreover, the MSI-H tumors derived from the serrated pathway are more common of the two, and frequently display excessive CpG island promoter hypermethylation (CIMP-high; Table 1). This promoter hypermethylation results in epigenetic silencing of a large number of tumor-suppressor genes, including *MLH1*, which causes the associated MSI-H phenotype. Somatic mutations typically include activating mutations in *BRAF* (V600E), and less commonly *KRAS* or aberrant EGFR activation, which occur during the early stages of serrated polyp development. In contrast to tubular adenomas, bilallelic inactivation of *APC* is not an initiating event in this pathway (Figure 1). Despite this molecular understanding and the development of novel drugs to target them, additional treatments are still needed given the inferior outcomes observed in *BRAF*-mutated colon cancers within the context of their microsatellite status.[1]

Discovery of high-risk genetic variants for this pathway represents a promising strategy to identify additional therapeutic targets for this subset of colorectal cancers. Epidemiologic data from families of serrated polyposis patients strongly suggest a heritable predisposition exists toward serrated colorectal carcinogenesis. First-degree relatives of serrated polyposis patients are at significantly higher elevated risk of developing serrated polyps themselves. In addition, elevated pleiotropic cancer risks are present in these families. An Australian cohort demonstrated that relatives are at an increased risk of pancreatic cancer.[2] First-degree relatives of those with MSI-H CRC who do not have Lynch syndrome or serrated polyposis are at increased risk (standardized incidence ratios) of developing stomach, ovarian, and liver cancers.[3]

Through exome sequencing of individuals who develop multiple sessile serrated polyps and/or serrated polyposis, we recently identified novel high-risk variants for the serrated pathway.[4] We demonstrated that such individuals are approximately fourfold enriched for rare, germline loss-of-function (LoF) mutations (defined as nonsense and splice-site mutations) in genes responsible for oncogene-induced senescence (OIS) mechanisms. OIS is a tumor-suppressive mechanism that is activated by the replicative and metabolic stress caused by oncogenic transformation. This hypothesis was based on the observations from genetic mouse models of serrated neoplasia in which the *BRAF* V600E mutation or activating *KRAS* mutation was alone sufficient to induce serrated neoplasia in the long-term; however, in the short-term, OIS barriers prevented rapid tumorigenesis.[5–7] Concurrent inactivation of these OIS mechanisms with activating *BRAF/KRAS* mutations greatly expedited serrated neoplasia formation. In humans, activation of these critical OIS pathways (ATM–ATR DNA damage pathway and p16-RB pathway) has been previously demonstrated to be relevant in colonic precursor lesions in addition to lesions in other tissue types. Several of the OIS genes identified in serrated polyposis patients in this study (*ATM*, *RBL1*, and *XAF1*) have been previously implicated in human or animal studies of colorectal carcinogenesis.

We next demonstrated that the remaining patients with serrated polyposis (who do not have an obvious loss-of-function mutation) actually harbor deleterious variants in genes previously unassociated, but critical to these OIS mechanisms. Cross-referencing all rare LoF mutations found in patients with an orthogonal database (not dependent on senescence characteristics) of all genes implicated in cancer by genome-wide association studies, we discovered two unrelated individuals with identical nonsense mutations in *RNF43,* a gene frequently mutated in mucinous neoplasms of the pancreas and stomach that encodes for a negative regulator of Wnt signaling through Wnt receptor endocytosis. This enrichment in cases of serrated polyposis was significant over controls with sizeable effect sizes (odds ratio 460, $P = 6.8 \times 10^{-5}$). Analysis of publicly available microarrays of sporadic serrated polyps and tubular adenomas, we found RNF43 to be significantly downregulated in the serrated pathway. Through functional experiments in pancreatic duct cells harboring the *KRAS* G12D oncogene, we demonstrated that silencing of *RNF43* impaired ATR–ATM DNA damage signaling in response to UV radiation, as evident by impaired phosphorylation of Chk1 and p53.

Subsequently, another group has further generalized the importance of RNF43 to the development of sporadic MSI-H colorectal cancers.[8] Performing whole-exome sequencing on 185 formalin-fixed, paraffin-embedded colon cancers from two Harvard cohorts, Giannakis *et al.* discovered deleterious somatic mutations in *RNF43* to be present in 18.9% of these cancers, in addition to being frequently mutated in endometrial cancers. Interestingly, 50% of the deleterious mutations discovered were frameshift mutations occurring at microsatellite loci within the gene. Validation of a small subset of tumors by next-generation sequencing or Sanger sequencing demonstrated an overall accuracy of 97% for the *RNF43* mutation calls made by software. To replicate these

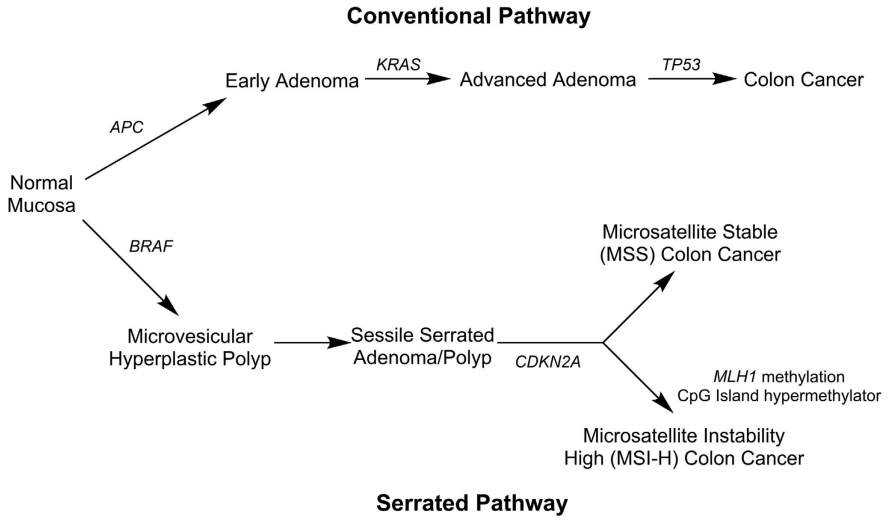

Figure 1 Serrated Pathway to Colorectal Cancer. Schematic comparing the mutational changes involved from normal mucosa to colorectal cancer. The top half represents the conventional pathway to colorectal cancer, with the most frequent mutational events described. The bottom half demonstrates common events in the serrated pathway.

Table 1 Important acronyms and definitions

Acronym	Definition
CIMP	**CpG Island Methylator Phenotype (high or low).** Excessive methylation of the CpG islands in gene promoters which results in transcriptional silencing. A panel of genes is used to determine this status.
MSI-H	**Microsatellite Instability High**. Errors at repeating nucleotides of 1-6 base pairs in length that may result in frameshift mutations. A panel of microsatellites determined by the National Cancer Institute, colloquially referred to as the Bethesda markers, is used to assess this trait.
MSS	**Microsatellite Stable**
OIS	**Oncogene-induced senescence.** A tumor-suppressive mechanism by which oncogene activation triggers a process to initiate cell growth arrest.

results, the authors reanalyzed 222 colorectal cancer cases from The Cancer Genome Atlas.[9] RNF43 mutations were present reliably in 17.6% of cases, and 49 cases were from the initial publication. The discrepancy between these results and previously published analyses of the TCGA data set may be attributable to newer algorithms in the detection of significant insertion and deletion events, which continue to be an ongoing challenge in their accuracy compared with single-nucleotide polymorphisms. Under prior methodologies, many true mutations at microsatellite sites were falsely discarded as errors due to their resemblance to sequencing artifacts caused by polymerase slippage during the exome enrichment step.

Consistent with the importance of this RNF43 in the serrated pathway, these mutations were particularly enriched in those tumors with MSI-H status, occurring in ~80% of this subset of colorectal cancers ($P < 2.2 \times 10^{-16}$, Fisher's exact test). Complementary to our initial microarray comparisons of sporadic polyps from the serrated and conventional pathway of colorectal carcinogenesis, somatic *RNF43* mutations appeared mutually exclusive with deleterious *APC* mutations.

The results from these studies have clinical consequences for epidemiology, genetic testing, and treatment strategies. First, these mutations provide firm genetic support for the multiple cancer risks observed in families and first-degree relatives of those afflicted with serrated polyposis. Many of the genes found in the primary mechanisms of OIS contain tumor-suppressor genes with already established pleiotropic effects. The discoveries of germline and somatic *RNF43* mutations in serrated lesions provide additional genetic evidence of such pleiotropy as the gene is found frequently mutated in gastric, pancreatic, ovarian, and endometrial cancers.

The germline mechanisms disrupted in serrated polyposis patients and the somatic mutations found in sporadic MSI-H tumors should promote clinical trials of newly developed chemotherapeutics for sporadic serrated colorectal tumors. Clinical trials of poly (ADP-ribose) polymerase inhibitors have demonstrated promise in individuals with germline mutations in upstream DNA damage repair pathways, notably those with *BRCA1* or *BRCA2* mutations, for individuals with breast or ovarian cancers.[10,11] Such agents push tumor cells with deficiencies in DNA repair pathways into mitotic catastrophe with the accumulation of double-stranded DNA breaks. The presence of germline and somatic *RNF43* mutations also confers the possibility of using an additional class of therapeutic agents. Recently, porcupine inhibitors, a new class of drugs that impair Wnt secretion, have demonstrated efficacy in slowing the tumor growth of pancreatic cancer cell lines that harbor deleterious somatic *RNF43* mutations.[12]

Despite the initial focus upon epigenetics due to the observed CIMP-high phenotype, the genetics of serrated neoplasia have a critical role in determining disease risk and therapeutic strategies. Additional experiments with larger cohorts of serrated polyposis will likely reveal additional high-risk genes given the broad genetic heterogeneity evident, leading to discovery of additional novel targets. In contrast to other tumor types with hypermethylation, the search for the inciting somatic mechanisms that trigger the widespread epigenetic changes observed in these cancers remains uncertain. These efforts over the upcoming years have the potential to further change preventative and therapeutic approaches toward colon cancer.

CONFLICT OF INTEREST

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