Gastroenterology 2014;146:520–529

Germline Mutations in Oncogene-Induced Senescence Pathways Are Associated With Multiple Sessile Serrated Adenomas

Manish K. Gala,[1] Yusuke Mizukami,[1,2,3] Long P. Le,[4] Kentaro Moriichi,[1] Thomas Austin,[1] Masayoshi Yamamoto,[1] Gregory Y. Lauwers,[4] Nabeel Bardeesy,[2] and Daniel C. Chung[1,2]

[1]Gastrointestinal Unit, Department of Medicine, and [4]Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; [2]Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, Massachusetts; and [3]Center for Clinical and Biomedical Research, Sapporo Higashi Tokushukai Hospital, Sapporo, Japan

BACKGROUND & AIMS: Little is known about the genetic factors that contribute to the development of sessile serrated adenomas (SSAs). SSAs contain somatic mutations in *BRAF* or *KRAS* early in development. However, evidence from humans and mouse models indicates that these mutations result in oncogene-induced senescence (OIS) of intestinal crypt cells. Progression to serrated neoplasia requires cells to escape OIS via inactivation of tumor suppressor pathways. We investigated whether subjects with multiple SSAs carry germline loss-of-function mutations (nonsense and splice site) in genes that regulate OIS: the p16-Rb and ATM-ATR DNA damage response pathways. **METHODS:** Through a bioinformatic analysis of the literature, we identified a set of genes that function at the main nodes of the p16-Rb and ATM-ATR DNA damage response pathways. We performed whole-exome sequencing of 20 unrelated subjects with multiple SSAs; most had features of serrated polyposis. We compared sequences with those from 4300 subjects matched for ethnicity (controls). We also used an integrative genomics approach to identify additional genes involved in senescence mechanisms. **RESULTS:** We identified mutations in genes that regulate senescence (*ATM*, *PIF1*, *TELO2*, *XAF1*, and *RBL1*) in 5 of 20 subjects with multiple SSAs (odds ratio, 3.0; 95% confidence interval, 0.9–8.9; $P = .04$). In 2 subjects, we found nonsense mutations in *RNF43*, indicating that it is also associated with multiple serrated polyps (odds ratio, 460; 95% confidence interval, 23.1–16,384; $P = 6.8 \times 10^{-5}$). In knockdown experiments with pancreatic duct cells exposed to UV light, RNF43 appeared to function as a regulator of ATM-ATR DNA damage response. **CONCLUSIONS:** We associated germline loss-of-function variants in genes that regulate senescence pathways with the development of multiple SSAs. We identified *RNF43* as a regulator of the DNA damage response and associated nonsense variants in this gene with a high risk of developing SSAs.

Keywords: Serrated Polyposis Syndrome; Hereditary Colon Cancer; Sessile Serrated Polyp; RNF43.

Sessile serrated adenomas (SSAs) are newly recognized precursor lesions to colorectal cancer. Found in 2% of average-risk individuals undergoing their first screening colonoscopy, SSAs are believed to give rise to sporadic microsatellite instability–high colon cancers and *BRAF*-mutated microsatellite stable colon cancers.[1,2] Despite their relative infrequency, serrated lesions are estimated to be responsible for approximately 20% to 35% of all colon cancers.[3,4] In contrast to tubular adenomas, SSAs are not dependent on mutations in the *APC*/β-catenin axis but instead exhibit somatic mutations in *BRAF*, or less commonly *KRAS*, early in their development.[4–6] To determine the presently unknown genetic susceptibility for this lesion, we chose to study subjects who develop multiple SSAs, a hallmark feature of serrated polyposis syndrome.

Although there is no clear consensus on the clinical definition of serrated polyposis syndrome, its prevalence has been estimated to be as high as 1:3000 in populations primarily of European ancestry.[7] Affected subjects have a lifetime risk of colon cancer as high as 54% and may have a strong personal or family history of extracolonic cancers.[8,9] Despite the broad phenotypic variability observed with this syndrome, the epidemiological evidence shows an underlying genetic risk. First-degree relatives have a 32% risk of developing multiple serrated polyps and a 5-fold increased risk of colon cancer.[10,11] More recently, an increased risk of pancreatic cancer has also been observed.[12] Attempts to model the genetics of this syndrome as monogenic among unrelated subjects or as Mendelian through linkage analyses have not been successful.[13,14] The genetic basis for this disorder remains undefined, with the exception that mutations in *MUTYH* have been observed in only a small minority of cases with concomitant tubular adenomas.[15]

Given this variable clinical presentation in an extreme phenotype and likely underlying genetic complexity and heterogeneity, we hypothesized that high-risk variants may be localized to specific pathways instead of a single gene. Whereas mice with inactivating mutations in *APC* rapidly develop tubular adenomas, *BRAF* or *KRAS* mutations that are associated with serrated polyps are alone insufficient to

Abbreviations used in this paper: CI, confidence interval; DDR, DNA damage response; GWAS, genome-wide associated studies; LoF, loss-of-function; OIS, oncogene-induced senescence; OR, odds ratio; SSA, sessile serrated adenoma.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2013.10.045

provoke intestinal tumorigenesis.[16,17] After a brief period of hyperproliferation, crypt cells undergo growth arrest due to metabolic and replicative stress, a process termed oncogene-induced senescence (OIS). Additional mutational events are required for neoplastic progression.[18] Accordingly, engineered bypass of OIS at the genetic level quickly recapitulates the serrated neoplasia phenotype found in humans.[17] In the human colon, OIS has been shown to be primarily mediated by the p16-RB– and ATM-ATR–mediated DNA damage response (DDR) pathways.[19,20] Because bypass of OIS is fundamental to progression of SSA, we sought to determine whether subjects with multiple SSAs are enriched with germline, strong loss-of-function (LoF) mutations (nonsense and splice site) in either of these 2 pathways.

Materials and Methods

Ethics Statement and Study Recruitment

This study was approved by the institutional review board of Massachusetts General Hospital under protocol no. 2011P001161. Written informed consent was obtained from all study participants. Twenty unrelated study participants were recruited from clinics in the Gastroenterology Division of Massachusetts General Hospital.

Given our focus on SSAs specifically and not serrated polyps in general, we enrolled subjects with a diagnosis of multiple SSAs who fulfilled a set of criteria that were modified from the World Health Organization criteria for serrated polyposis to define a robust genotype-phenotype relationship for SSAs (Table 1).[21] We exclusively counted SSAs regardless of size toward multiplicity thresholds despite the existence of other types of serrated polyps in study participants. A family history of serrated polyposis was expanded to include a family history of colorectal cancer given the strong associated genetic risk and the absence of SSAs from older pathology reports.[22] Participants were excluded if they had a previous diagnosis of a known polyposis syndrome through genetic testing. However, no subjects were excluded as a result of a positive genetic test.

Given that all study participants were of complete (95%) or partial (5%) European descent, we used the exomes of 4300 European Americans from the National Heart, Lung, and Blood Institute Exome Sequencing Project as genetic controls.[23] No polyp or cancer histories are available for these subjects and were assumed to be negative to bias the study toward the null hypothesis.

Table 1. Enrollment Criteria

Revised World Health Organization criteria for serrated polyposis	Enrollment criteria
1. At least 5 serrated polyps proximal to the sigmoid colon, with 2 or more >10 mm	1. At least 5 SSAs proximal to the sigmoid colon
OR	OR
2. Any number of serrated polyps proximal to the sigmoid colon in a subject who has a first-degree relative with serrated polyposis	2. Any number of SSAs proximal to the sigmoid colon in a subject with a first-degree relative with sessile serrated polyposis or colon cancer
OR	OR
3. >20 serrated polyps of any size but distributed throughout the colon	3. >20 SSAs but distributed throughout the colon

NOTE. Fulfillment of any of the 3 criteria is sufficient for diagnosis or enrollment. Polyp counts are intended to be cumulative over time. Examples of other types of serrated polyps include traditional serrated adenomas and microvesicular-type hyperplastic polyps.

Gene Set Construction

We prospectively defined a gene set for the p16-Rb and ATM-ATR DDR pathways for which strong LoF mutations could impair OIS. Core components of the p16-Rb axis in which loss would result in impairment were previously defined as *CDKN2A*, *CDKN2B*, *CDKN2C*, *CDKN2D*, *CDKN1A*, *CDKN1B*, *RB1*, *RBL1*, and *RBL2*.[24] Given the variable impact according to cell type of the numerous downstream effectors of the ATM-ATR–mediated DDR on OIS, we defined the critical nodes for this pathway as all genes in which loss results in diminished activation or function of DNA checkpoint kinases: ATM, ATR, Chk1, or Chk2. Although p53 has been shown to be a critical downstream effector of senescence in other cell types, multiple animal studies have shown that biallelic loss of p53 fails to increase intestinal polyp multiplicity or accelerate progression of early-stage conventional adenomas or serrated polyps.[17,18,25] As such, we did not incorporate p53 into our analysis.

A python script was devised to extract all 19,049 HUGO Gene Nomenclature Committee (HGNC) gene symbols of protein coding genes and all associated one-word gene synonyms from the ExPASy GPSDB Gene/Protein Synonyms finder (http://gpsdb.expasy.org/#). Subsequently, this script used the e-utilities function of PubMed (http://www.ncbi.nlm.nih.gov/books/NBK25500/) to cross-reference each gene name and its synonyms sequentially against ATM, ATR, Chk1, and Chk2. All search terms were coded as text words. This search algorithm yielded 313,747 PubMed IDs last scanned as of December 11, 2012. Each title, abstract, and/or publication was manually reviewed. This analysis yielded 224 genes in which loss impaired activation or function of ATM, ATR, Chk1, or Chk2 (Supplementary Table 1). In addition to the 9 genes from the p16-RB pathway, the final gene set consisted of 233 genes for genes relevant to OIS pathways in the colon.

Genes containing cancer-related genome-wide association studies (GWAS) loci ($P < 1.0 \times 10^{-5}$) were obtained from the National Human Genome Research Institute Catalog of Genome-wide Association Studies (http://www.genome.gov/gwastudies/), which assigned loci to the closest gene if applicable. The following search terms were used to compile a list of 555 genes containing such loci: cancer, tumor, leukemia, lymphoma, sarcoma, melanoma, myeloma, rhabdomyosarcoma, and schwannoma (Supplementary Table 2).

Exome Sequencing

Genomic DNA was isolated from peripheral blood or from mouthwash samples if study participants harbored active hematopoietic neoplasms (QIAmp; Qiagen Inc, Valencia, CA). Exome sequencing was performed by Otogenetics Corp (Norcross, GA).