Research Article

Cancer Prevention Research

Gene Signature in Sessile Serrated Polyps Identifies Colon Cancer Subtype

Priyanka Kanth[1], Mary P. Bronner[2,3], Kenneth M. Boucher[3,4], Randall W. Burt[1,3], Deborah W. Neklason[5], Curt H. Hagedorn[1,6], and Don A. Delker[1]

Abstract

Sessile serrated colon adenoma/polyps (SSA/P) are found during routine screening colonoscopy and may account for 20% to 30% of colon cancers. However, differentiating SSA/Ps from hyperplastic polyps (HP) with little risk of cancer is challenging and complementary molecular markers are needed. In addition, the molecular mechanisms of colon cancer development from SSA/Ps are poorly understood. RNA sequencing (RNA-Seq) was performed on 21 SSA/Ps, 10 HPs, 10 adenomas, 21 uninvolved colon, and 20 control colon specimens. Differential expression and leave-one-out cross-validation methods were used to define a unique gene signature of SSA/Ps. Our SSA/P gene signature was evaluated in colon cancer RNA-Seq data from The Cancer Genome Atlas (TCGA) to identify a subtype of colon cancers that may develop from SSA/Ps. A total of 1,422 differentially expressed genes were found in SSA/Ps relative to controls. Serrated polyposis syndrome ($n = 12$) and sporadic SSA/Ps ($n = 9$) exhibited almost complete (96%) gene overlap. A 51-gene panel in SSA/P showed similar expression in a subset of TCGA colon cancers with high microsatellite instability. A smaller 7-gene panel showed high sensitivity and specificity in identifying *BRAF*-mutant, CpG island methylator phenotype high, and *MLH1*-silenced colon cancers. We describe a unique gene signature in SSA/Ps that identifies a subset of colon cancers likely to develop through the serrated pathway. These gene panels may be utilized for improved differentiation of SSA/Ps from HPs and provide insights into novel molecular pathways altered in colon cancer arising from the serrated pathway. *Cancer Prev Res; 9(6); 456–65. ©2016 AACR.*

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

Introduction

Colon cancer is the second leading cause of cancer-related deaths in United States and third most common cancer in men and women (1). Serrated colon polyps are found in 12% to 36% of patients undergoing routine screening colonoscopy (2–4). Serrated polyps are classified into three groups: hyperplastic polyps (HP), sessile serrated adenoma/polyps (SSA/P), and traditional serrated adenomas (TSA; ref. 5). Both SSA/Ps and relatively rare TSAs have malignant potential. Histologically, SSA/Ps often have basilar crypt dilation, which may present as an L-shaped or inverted T-shaped morphology. HPs lack these specific features (6). However, differentiating SSA/Ps from HPs by colonoscopy or histopathology remains difficult due to overlapping morphologic and pathologic features (7, 8).

The serrated polyposis syndrome (SPS) is an extreme phenotype, with patients presenting with multiple SSA/Ps, and has a high risk of colon cancer (9–11). So far, no inherited gene mutation has been found in SPS. The risk of SSA/Ps progressing to colon cancer is not unique to SPS patients and has also been described in patients with sporadic SSA/Ps (2, 12).

The "serrated polyp pathway" has been described as an underlying mechanism in the development of colon cancer from SSA/Ps and may account for 20% to 30% of sporadic colon cancers (6, 13–15). However, the molecular mechanisms or signaling pathways important in the progression of SSA/Ps to colon cancer are uncertain. DNA microsatellite instability (MSI), CpG island methylation, and *BRAF* mutations are possible underlying molecular mechanisms in the development of SSA/Ps (14–17). At least a subset of proximal colorectal cancers have the CpG island methylator phenotype (CIMP) and high MSI (MSI-H), suggesting similar molecular backgrounds in serrated polyps and proximal cancer (18).

There is limited information on gene expression profiles differentiating SSA/Ps from traditional HPs. Two prior studies have described gene expression in SSA/Ps using microarray technologies (19, 20). We recently identified >1,200 differentially expressed genes in SSA/Ps from patients with SPS using RNA sequencing (RNA-Seq) and developed several immunohistochemical markers specific for SSA/Ps (21). However, comprehensive RNA-Seq gene expression profiles have not been defined for sporadic SSA/Ps and HPs, and it is not known whether sporadic SSA/Ps differ from syndromic SSA/Ps that have a very high risk for progressing to colon cancer. The goals of our study were 2-fold:

[1]Department of Gastroenterology, University of Utah, Salt Lake City, Utah. [2]Department of Pathology, University of Utah, Salt Lake City, Utah. [3]Huntsman Cancer Institute, Salt Lake City, Utah. [4]Division of Epidemiology, University of Utah, Salt Lake City, Utah. [5]Division of Genetic Epidemiology, University of Utah, Salt Lake City, Utah. [6]The Central Arkansas Veterans Healthcare System and University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

C.H. Hagedorn and D.A. Delker share senior authorship of this article.

Corresponding Author: Priyanka Kanth, Department of Gastroenterology, University of Utah Health Care, 30 N 1900 East, School of Medicine 4R118, Salt Lake City, UT 84132. Phone: 801-587-3863; Fax: 801-581-7476; E-mail: priyanka.kanth@hsc.utah.edu

doi: 10.1158/1940-6207.CAPR-15-0363

©2016 American Association for Cancer Research.

first, to identify a panel of differentially expressed genes that discriminate between SSA/P's and HP's, and second, to characterize a subset of SSA/P genes that are also differentially expressed in colon cancers that likely develop through the serrated pathway. We compared gene expression in prospectively collected SSA/Ps from patients with SPS and sporadic SSA/Ps, HPs, tubular adenomas, and normal colon tissue to identify uniquely expressed genes in SSA/Ps. We report a 51-gene signature that differentiates SSA/Ps from HPs and shares a similar transcriptional profile with a subtype of colon cancers that may develop through the serrated pathway. Furthermore, our findings describe a novel 7-gene panel differentially expressed in SSA/Ps that has both high sensitivity and specificity for detection of *BRAF*-mutant, CIMP high (CIMP-H), and *MLH1*-silenced colon cancers.

Materials and Methods

Patients

Samples were obtained from patients visiting University of Utah Health Care and George Wahlen Veterans Affairs Medical Center (Salt Lake City, UT) between ages 45 and 75 for routine screening, surveillance, or diagnostic colonoscopy. Patients with SPS were between 18 to 75 years of age. Subjects with family history of colon cancer, familial cancers, including familial adenomatous polyposis and Lynch syndrome, history of inflammatory bowel disease, and prior colonic resections were excluded. The samples were prospectively collected from 2008 to 2013 for RNA-Seq. All patients signed and agreed to informed consent as approved by the respective hospitals Institutional Review Boards. If polyps were found during colonoscopy, a biopsy of polyp tissue was collected in formalin for histopathologic diagnosis. If additional polyp tissue remained, a small biopsy of polyp tissue was collected in RNAlater for RNA-Seq. If a polyp was too small to obtain a biopsy for both histology and RNA-Seq, a tissue sample for RNA-Seq was not collected for the study.

Twelve sessile serrated polyps were obtained from 8 patients with serrated polyposis syndrome (ten right colon and two left colon; refs. 21). SSA/Ps from these patients were previously analyzed for specific mRNA changes by qPCR but not analyzed by RNA sequencing. Uninvolved mucosa from right and left colon was also collected. Right colon was defined as colonic region from splenic flexure to cecum.

Sporadic sessile serrated polyps ($n = 9$, six right colon, three left colon), HPs ($n = 10$, two right colon, eight left colon), and adenomatous polyps ($n = 10$, nine right colon, one left colon) were obtained along with uninvolved mucosa from patients undergoing routine colonoscopy. Normal colon tissue ($n = 20$, ten right colon, ten left colon) was obtained from patients undergoing screening colonoscopy, with no polyps found on exam. All samples were collected prospectively and placed in RNAlater (Invitrogen) immediately after tissue removal, stored at 4°C overnight, and then at −80°C prior to performing RNA isolation. The demographics of sporadic SSA/Ps and hyperplastic polyps are presented in Supplementary Table S1A and B, respectively. The demographics of patients with adenoma and control colon tissues (analyzed using qPCR) have been described in our prior publication (21). Four retrospectively obtained frozen colon cancer samples (three right colon, one left colon) obtained from the University of Utah (Salt Lake City, UT) tissue bank were also sequenced.

Pathologic classification

All biopsy specimens were reviewed by an expert gastrointestinal pathologist. Serrated polyps were classified according to the recent recommendations of the Multi-Society Task Force on Colorectal Cancer for post-polypectomy surveillance and as described previously (21, 22). HPs were not subdivided into microvesicular hyperplastic polyps (MVHP) and goblet cell hyperplastic polyps, as these classifications are not used clinically or discussed in the recent post-polypectomy colonoscopy surveillance guidelines (22). We decided to follow the classification that is most appropriate and practical in clinical practice with the aim to define clinically relevant and realistic gene signatures. Moreover, these two HP subtypes have not been shown to have different risks for the development of colon cancer.

RNA isolation, RNA-Seq, and differential expression analysis

Total RNA was isolated using TRIzol (Invitrogen) and quality of RNA assessed by an Agilent 2000 Bioanalyzer as described previously (21, 23, 24). RNA-Seq was performed on 86 individual colon samples: 21 SSA/Ps (12 syndromic and 9 sporadic), 10 HPs, 10 adenomatous polyps, 21 uninvolved colon, 20 control colon, and 4 colon cancer samples. PCR-amplified cDNA sequencing libraries were prepared using oligo dT–selected RNA according to the Illumina TruSeq library protocol. Single-end 50 bp sequence reads were performed on an Illumina HiSeq 2000 instrument and aligned to the GRCh37/Hg19 human reference genome using the NovoAlign (Novocraft) application as described previously (21). Differentially expressed genes were determined using the USeq DefinedRegionsDifferentialSeq application and hierarchical clustering and principal component analysis of genes and samples performed using Cluster 3.0 as described previously (21). The RNA-Seq datasets described in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) with accession number GSE76987.

Derivation of 51 SSA/P gene signature and 7-gene panel

A 27-gene signature was developed to include genes with high fold change expression in SSA/Ps compared with HPs (see Supplementary Table S2). A separate 28-gene signature was obtained using a leave-one-out cross-validation method (see selection and cross-validation of a 28-gene signature section below). Combining the two gene signatures (27 and 28) resulted in a 55-gene signature unique for SSA/Ps. Four of these 55 genes were not found in colon cancer RNA-Seq datasets from the TCGA database, resulting in a 51-gene signature to compare across all RNA-Seq datasets. We next looked at the correlation of increased expression of each of the 51 genes in *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers (see Supplementary Tables S3 and S4). Seven of the 51 genes (*ZIC5*, *SEMG1*, *TRNP1*, *MUC6*, *CRYBA2*, *FSCN1*, and *ZIC2*) frequently overexpressed in MSI-H colon cancers were also frequently overexpressed in *BRAF*-mutant, CIMP-H, and/or *MLH1*-silenced colon cancers. We used this 7-gene panel for sensitivity and specificity calculations for identifying colon cancers that likely develop through the serrated pathway.

Selection and cross-validation of a 28-gene signature

Sequencing data from 10 HP and 21 SSA/P samples were used to construct and cross-validate a gene signature. Prior to analysis, genes differentially expressed between left and right colon (≥2-fold change, FDR < 0.01) were removed. An

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

"unpaired" analysis was then performed on all 31 serrated polyp samples using DESeq2 negative binomial statistics with histology as the only predictor. The FDR threshold for the signature genes was set at 0.01. Twenty-eight genes met these criteria and were used for cross-validation. The average of log (count + 0.5) for the selected genes was used to form separate signatures for HP and serrated colon polyps samples. A normalized Euclidean distance measure was constructed from the selected genes. SDs < 0.05 were increased to 0.05 in the normalization so that genes with unrealistically low variability did not exert excess influence on the signature (25). The signature for each class is represented by the geometric average, or centroid, of the class. Samples are predicted to be in the class with the closest centroid. To evaluate the signature, the entire process of selection of the genes to form the signature, construction of the centroid for each class, calculation of the Euclidean distance measure, and classification was cross-validated. A principal component analysis was performed using Cluster 3.0 and a 3D plot constructed using the "rgl" package in R.

Analysis of signature genes in published microarray data of serrated polyps

No previously published RNA-Seq data of serrated polyps are available for comparison with our datasets. We evaluated the expression of each of our 51 signature genes in a previously published microarray dataset (GEO number GSE43841; ref. 19). See Supplementary Methods.

Comparison with TCGA colon cancer RNA-Seq datasets

Fifty-one SSA/P signature genes were used to interrogate 68 colon cancer RNA-Seq datasets from TCGA [36 specimens from Christiana Healthcare and 32 from Memorial Sloan Kettering (New York, NY)] and four from the University of Utah (26). Raw sequencing data for each colon cancer dataset was downloaded from the TCGA database (27) and normalized by number of transcript reads per kilobase of gene length per million of total reads (RPKM). There was expression data for 18,130 unique RefSeq genes in both the TCGA and University of Utah RNA-Seq datasets. A total of 195 TCGA colon cancer datasets were also evaluated for mRNA expression in the 51 signature genes using the cBioPortal for Cancer Genomics (28, 29).

Mutual exclusivity and cooccurrence analysis

Mutual exclusivity and cooccurrence of genomic alterations in each of our 51 signature genes and incidence of *BRAF* mutations was evaluated using the cBioPortal for Cancer Genomics. This analysis uses a previously published statistical method, mutual exclusivity modules, to identify genes that may be involved in the same cancer pathway (30).

Sensitivity and specificity of a 7-gene panel

The sensitivity and specificity of a 7-gene panel was evaluated in 182 TCGA colon cancer samples with gene expression, methylation, and *BRAF* mutation data available. There were 31 *MLH1*-silenced, CIMP-H, and/or *BRAF*-mutant samples out of 182 regarded as positive and the rest as negative. Cut-off values for each gene were set at twice the average expression of all samples. K-fold cross-validation was used to get an estimate of sensitivity and specificity. In addition to individual expression, we also investigated panels of genes. For the panels, we considered the count of the number of genes above the 2-fold threshold as a predictor (see details in Supplementary Methods). PCR validation was performed on 4 of these genes *FSCN1*, *ZIC5*, *SEMG1*, and *MUC6* (see Supplementary Methods).

Results

Differential gene expression analysis

RNA-Seq was performed on 86 colon specimens with a mean sequence depth of 14.7 million mapped reads per sample. Comparing syndromic ($n = 12$) and sporadic ($n = 9$) SSA/P RNA-Seq datasets to control right colon ($n = 10$), we identified 1,422 differentially expressed annotated genes ($\geq$2-fold change, FDR < 0.05) by negative binomial statistical analysis (Fig. 1A; Supplementary Table S2). Comparing HPs ($n = 10$) to control left colon ($n = 10$), we identified 711 differentially expressed genes using the same fold change and FDR cut-off value. A total of 475 genes were differentially expressed in both SSA/Ps and HPs. In the RNAs that were differentially expressed in SSA/Ps, 1,095 (77%) were protein coding and 327 (23%) were non-coding (Fig. 1B). A similar percentage of protein coding (80%) and non-coding (20%) RNAs was also significantly differentially expressed in HPs relative to control colon.

To determine if sporadic SSA/Ps had a gene expression profile similar to syndromic SSA/Ps, we compared differentially

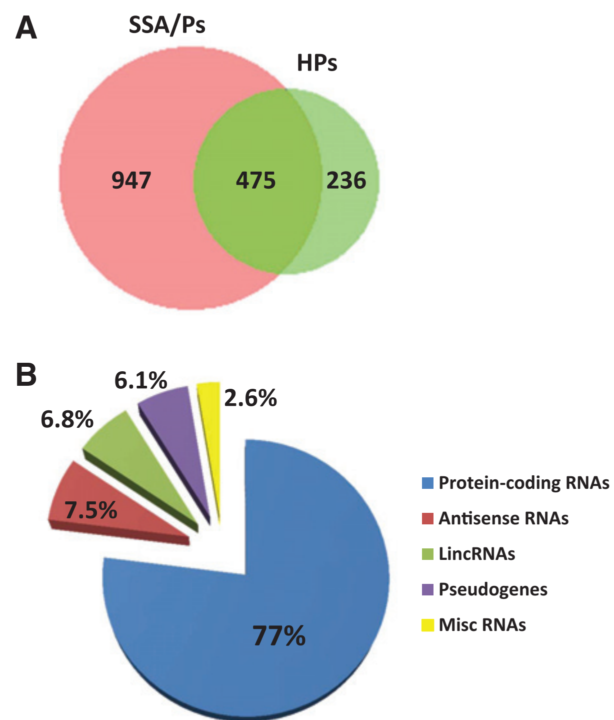

Figure 1.
Differentially expressed annotated protein-coding and non-coding RNAs in SSA/Ps and traditional HPs identified by RNA-Seq. A, differentially expressed genes with a $\geq$2-fold change and FDR < 0.05 in SSA/Ps ($n = 12$ for syndromic and $n = 9$ for sporadic) compared with control right colon ($n = 10$) and HPs ($n = 10$) compared with control left colon ($n = 10$). B, relative abundance of protein-coding and non-coding RNAs differentially expressed in SSA/Ps. Non-coding RNAs included antisense non-coding RNAs, long intergenic non-coding RNAs (lincRNAs), pseudogenes, and other miscellaneous (Misc) RNAs, including immunoglobulin and intronic RNAs.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

expressed genes with a ≥2- and 4-fold change in each group (Supplementary Fig. S1A and Fig. 2A, respectively). Greater than 89% (≥2 fold) and 96% (≥4 fold) of the differentially expressed genes observed in sporadic SSA/Ps were also differentially expressed in syndromic SSA/Ps. We are not aware of another gene expression comparison of sporadic and syndromic SSA/Ps, and these results describe major molecular similarities in SSA/Ps from these two very different patient cohorts. A total of 215 genes (77%) were uniquely differentially expressed ≥4-fold in SSA/Ps as compared with HPs (Fig. 2A), whereas nearly 86% of the differentially expressed genes in HPs overlapped with SSA/Ps and only 10 genes (14%) were uniquely differentially expressed ≥4-fold in HPs. This suggests that the molecular phenotype in HPs (considered at little or no risk for progression to colon cancer) is surprisingly similar to that of SSA/Ps (considered high risk). One notable difference between SSA/Ps and HPs was the magnitude of fold change in many differentially expressed genes. Hierarchical clustering of 27 protein-coding genes with average increased expression >13 fold in SSA/Ps illustrates what was shared in gene expression changes among all but two of the SSA/Ps (Fig. 2B; Supplementary Table S2). It should be noted that 2 of 10 (20%) HPs and 5 of 21 (24%) SSA/Ps were from right and left colon, respectively. Although our numbers of HPs from right colon and SSA/Ps from left colon are small, we did not see appreciable differences in gene expression between left and right HPs or SSA/Ps. Increased expression of these 27 genes was not observed in adenomatous polyp RNA-Seq datasets (Fig. 2C).

We also compared gene expression in the uninvolved colon ($n = 10$) of SPS patients and patients with sporadic SSA/Ps, with the control right colon ($n = 10$) of patients undergoing screening colonoscopy with no polyps (Supplementary Fig. S2). Surprisingly, 1,922 genes were differentially expressed between the uninvolved colon of patients with SSA/Ps and control colon (≥2-fold change, FDR < 0.01). A significant overlap in the gene expression profile of uninvolved colon from patients with SPS and sporadic SSA/Ps was observed. However, the magnitude of fold change was small for most genes (<3 fold), and the genes differentially expressed were not common to genes differentially expressed in SSA/Ps.

Selection and cross-validation of a gene signature that differentiates SSA/Ps from HPs

Count data from 31 serrated polyps (21 SSA/Ps and 10 HPs) were used in a leave-one-out cross-validation analysis. Twenty-eight genes with an FDR < 0.01 and ≥2-fold change (SSA/Ps vs. HPs) defined the signature (Supplementary Table S2). Twenty-eight of 31 serrated polyps were classified correctly for a nominal error rate of 10%. After cross validating four times, the cross-validated error rate was 18%. Principal component analysis of the gene expression of each of the 28 genes in all 31 serrated polyps is shown in Fig. 3A, which demonstrates the misclassification of two SSA/Ps and one HP. The relative expression of each of the 28 genes in SSA/Ps and HPs is shown in Fig. 3B. Six genes were overexpressed and 22 underexpressed in SSA/Ps relative to HPs.

Evaluation of gene signature in published microarray data of serrated polyps

We compared the relative expression of each of our 51-gene signature in SSA/Ps, MVHPs and normal colon (left and right) from a previously published microarray study (19). Clear separation of SSA/Ps from MVHPs and control colon was observed by hierarchical clustering (Supplementary Fig. S3). In fact, 5 of 6 MVHPs, showed gene expression patterns more closely resembling control colon than SSA/Ps.

Identifying colon cancers with the SSA/P gene signature in TCGA

We compared our 51-gene SSA/P signature with 68 colon cancer RNA-Seq datasets available in TCGA and four colon cancers obtained from the University of Utah (Fig. 4A; Supplementary Table S2). RNA-Seq data from 4 of the 55 genes were not available in the TCGA datasets. We performed RNA sequencing on four colon cancers from the University of Utah to identify potential laboratory/batch effect differences in gene expression between our RNA-Seq datasets and the TCGA datasets. The 51-gene SSA/P signature showed similar expression patterns between syndromic and sporadic SSA/Ps and the MSI-H subset of colon cancers. No batch effects were observed between our colon cancer datasets and the TCGA datasets. Sixty-three of 72 cancers had data on their MSI status, with 11 cancers being MSI-H (MSI status unknown for 9 colon cancers). Eighteen colon cancers clustered with SSA/Ps and 8 of the 18 colon cancers (44%) were MSI-H. This is a significant finding, as of the remaining 54 colon cancers that did not cluster with SSA/Ps, only 3 were MSI-H (6%). This suggests that our SSA/P signature identifies MSI-H cancers.

We also evaluated mRNA expression of each of our 51 SSA/P signature genes in 195 TCGA colon cancers using the cBioPortal for Cancer Genomics. Thirteen of the 51 signature genes had frequent increased mRNA expression in ≥10% of hypermutated colon cancers but not in nonhypermutated cancers (Table 1). Seven of these genes (*FSCN1*, *ZIC2*, *ZIC5*, *CRYBA2*, *MUC6*, *TRNP1*, and *SEMG1*) had increased mRNA expression in 13% to 30% of hypermutated and only 0% to 3% of nonhypermutated colon cancers with Fischer exact $P < 0.01$ (Table 1). Twenty-two of the 30 (73%) hypermutated colon cancers showed increased expression of at least one of the 7-gene panel. Seventeen of the 22 (77%) hypermutated colon cancers showing increased expression of at least one of the 7-gene panel also showed *MLH1* silencing. (Fig. 4B). Eleven of 51 genes showed frequent overexpression in CIMP-H and/or *MLH1*-silenced colon cancers, including all 7 that showed frequent increased expression in hypermutated cancers (Supplementary Table S3). We did not observe frequent increased expression of previous SSA/P markers (Annexin A10, *ANXA10* and claudin 1, *CLDN1*) in hypermutated, CIMP-H and/or *MLH1*-silenced colon cancers (Table 1 and Supplementary Table S3; refs. 19, 31).

Mutual exclusivity and cooccurrence analysis

Using the cBioPortal, we evaluated concurrent genomic alterations (RNA expression and somatic mutation) in each of our 51-gene panel and two genes from previous microarray studies (*ANXA10* and *CLDN1*) with alterations in *BRAF* (19, 31). Thirteen of 51 genes showed statistically significant associations with *BRAF* mutation both by Fisher exact test and log OR (Supplementary Table S4). Six of these genes (*FSCN1*, *ZIC5*, *CRYBA2*, *MUC6*, *TRNP1*, and *SEMG1*) were common to genes frequently overexpressed in hypermutated, CIMP-H and *MLH1*-silenced colon cancers. *ZIC2* and *CLDN1* did not show significant associations with *BRAF* mutation, and *ANXA10* showed a positive association by log OR but not the Fisher exact test.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

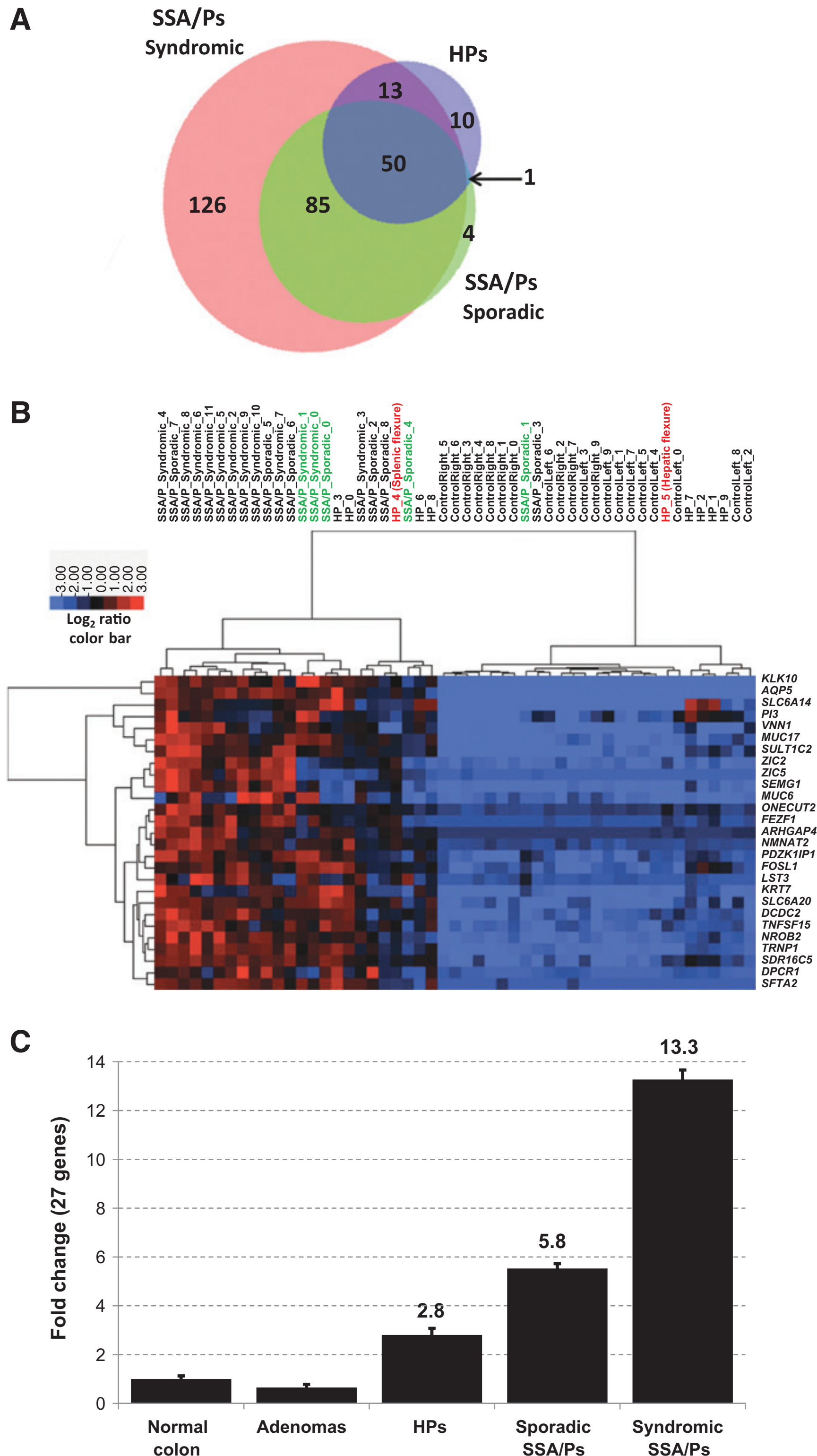

Figure 2.
Differentially expressed genes in syndromic and sporadic SSA/Ps and HPs by RNA-Seq. A, genes with ≥4-fold change and FDR < 0.05 in syndromic SSA/Ps ($n = 12$), sporadic SSA/Ps ($n = 9$), and HPs ($n = 10$). Syndromic and sporadic SSA/Ps were compared with control right colon, and HPs were compared with control left colon. B, relative expression of 27 protein-coding genes in syndromic SSA/Ps, sporadic SSA/Ps, HPs, and control left and right colon. Log_2 ratios comparing each individual sample with the mean of all samples were used for hierarchical clustering. Two right-sided HPs are labeled in red, and five left-sided SSA/Ps are labeled in green. C, mean fold change expression of the same 27 protein-coding genes described in B in normal colon, adenomas, HPs, and sporadic and syndromic SSA/Ps.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

Sensitivity and specificity of a 7-gene panel

Using a 7-gene panel (*FSCN1*, *ZIC2*, *ZIC5*, *CRYBA2*, *MUC6*, *TRNP1*, and *SEMG1*), we determined the sensitivity and specificity of each gene in identifying 31 *BRAF*-mutant, CIMP-H and/or *MLH1*-silenced colon cancers out of 182 total colon cancers from the TCGA database (Table 2A). The specificity of each gene in identifying this subset of cancers was very high, between 85% and 99%. SSA/P RNA markers *ANXA10* and *CLDN1* showed similar

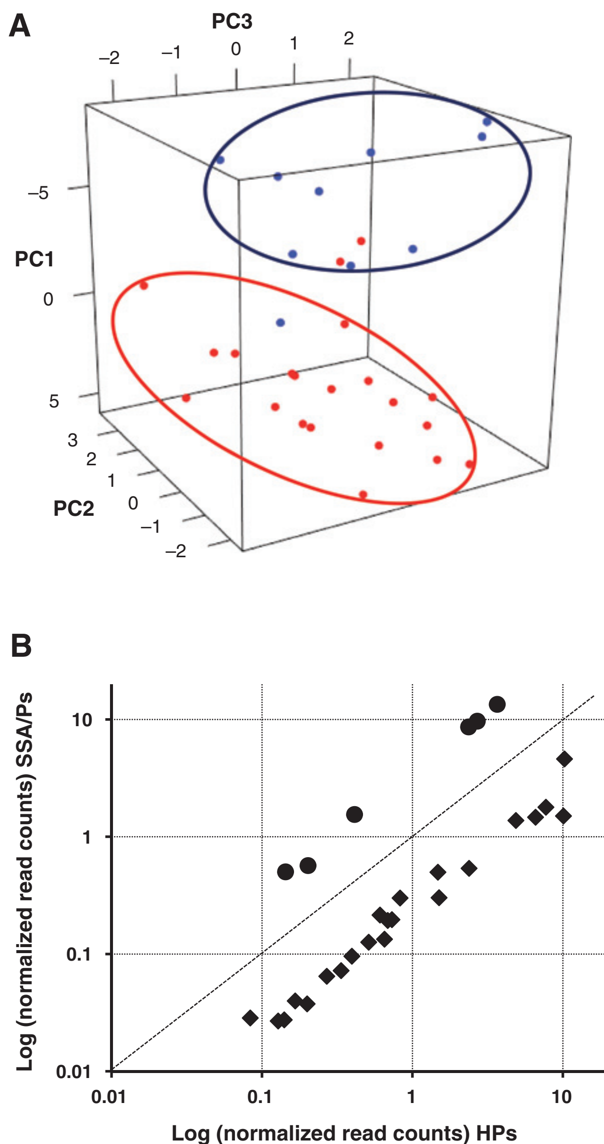

Figure 3.
Evaluation of a 28-gene signature to distinguish SSA/Ps from HPs. The 28-gene panel was developed using a leave-one-out cross-validation approach on 31 independent serrated polyps (21 SSA/Ps and 10 HPs) samples. A, principal component analysis of the 28-gene $\log_2$ ratios for each individual serrated polyp compared with the mean of all serrated polyps. Principal component 1 (PC1) accounted for 28% of the variation in the data and separated most SSA/Ps (red) from HPs (blue). Twenty-eight of 31 serrated polyps (~90%) clustered correctly similar to the nominal error rate were found in the cross-validation results. B, relative expression (log of normalized reads (RPKM) of the same 28 genes described in A in SSA/Ps and HPs. Six genes (circles) were overexpressed in SSA/Ps compared with HPs (range 2.8 to 3.7 fold), and 22 genes (squares) were underexpressed in SSA/Ps compared with HPs (range −2.2 to −6.7).

specificity to our 7-gene panel. In contrast, the sensitivity of each gene in identifying *BRAF*-mutant, CIMP-H and/or *MLH1*-silenced colon cancers was more variable between genes (26%–68%), with *ZIC5* showing the highest sensitivity at 68%. The two previously identified RNA markers for SSA/Ps were lower, with 19% and 6% sensitivity for *ANXA10* and *CLDN1*, respectively. Using a 7-gene panel, our sensitivity increased to 94% if at least one of the seven genes showed a 2-fold increase in expression (Table 2B). Using *ANXA10* or *CLDN1* with our 7-gene panel, the sensitivity was 97% and 94%, and the specificity was 72% and 63%, respectively (Supplementary Table S5). qPCR validation was performed on 4 genes (*FSCN1*, *ZIC5*, *SEMG1*, and *MUC6*) and showed high expression in SSA/Ps compared with HPs, uninvolved or control colon consistent with our RNA-Seq data (Supplementary Fig. S4).

Discussion

SSA/Ps are now recognized as polyps with malignant potential, with SSA/Ps originating in the serrated polyposis syndrome having the highest risk for progression to colon cancer. Recent cancer surveillance guidelines recommend earlier follow-up for patients with sporadic SSA/Ps almost at par with individuals with adenomatous polyps (22). Nevertheless, differentiating SSA/Ps from HPs by histopathology and identifying patients with SSA/Ps have some challenges in clinical practice. The RNA-Seq datasets we describe identify 51 differentially expressed genes in SSA/Ps that molecularly distinguish them from HPs. These genes are also differentially expressed in sporadic microsatellite unstable (MSI-H) colon cancers. We further refined our panel to seven genes that also show frequent overexpression in *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers. Our data provide clear evidence that RNA expression changes in *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers are observed in early SSA/Ps and that these new gene expression markers may lead to improved diagnostics for SSA/Ps. Moreover, our data demonstrate similar gene expression profiles of SSA/Ps in the SPS and sporadic SSA/Ps, indicating that common mechanisms of progression to cancer are operating in both.

Comparing the transcriptome of SSA/Ps and HPs produced findings that raise some critical questions about these two subtypes of serrated polyps with very different potentials for progression to colon cancer. It is unclear whether serrated adenocarcinoma originates directly through SSA/Ps or whether genetic alterations in certain hyperplastic polyps found in right colon lead to the development of SSA/Ps and eventually to colon cancer. SSA/Ps, especially in the SPS, have a significant risk for progression to cancer (9–11), whereas HPs have a negligible risk (32, 33). The finding that most of the genes found differentially expressed in HPs were also found in SSA/Ps at least partly explains why both types of polyps have a similar morphologic appearance. On the other hand, there were many uniquely and highly differentially expressed genes in SSA/Ps compared with HPs. The unique SSA/Ps gene signature established in this study provides an opportunity to identify critical pathways that may explain these differences in cancer risk.

Our 7-gene panel (*FSCN1*, *ZIC2*, *ZIC5*, *CRYBA2*, *MUC6*, *TRNP1*, and *SEMG1*) identified *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers with high sensitivity and specificity. In comparison with other gene markers described for SSA/Ps (*ANXA10* and *CLDN1*), our 7-gene panel showed increased sensitivity and similar specificity. This increase in sensitivity might be related to the use of RNA-Seq versus microarray technology. RNA-Seq provides a more quantitative analysis of transcript abundance and is not dependent on previously defined gene annotation. Also, the analysis of SSA/Ps from SPS patients, known to have high colon cancer risk, may have further increased our

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

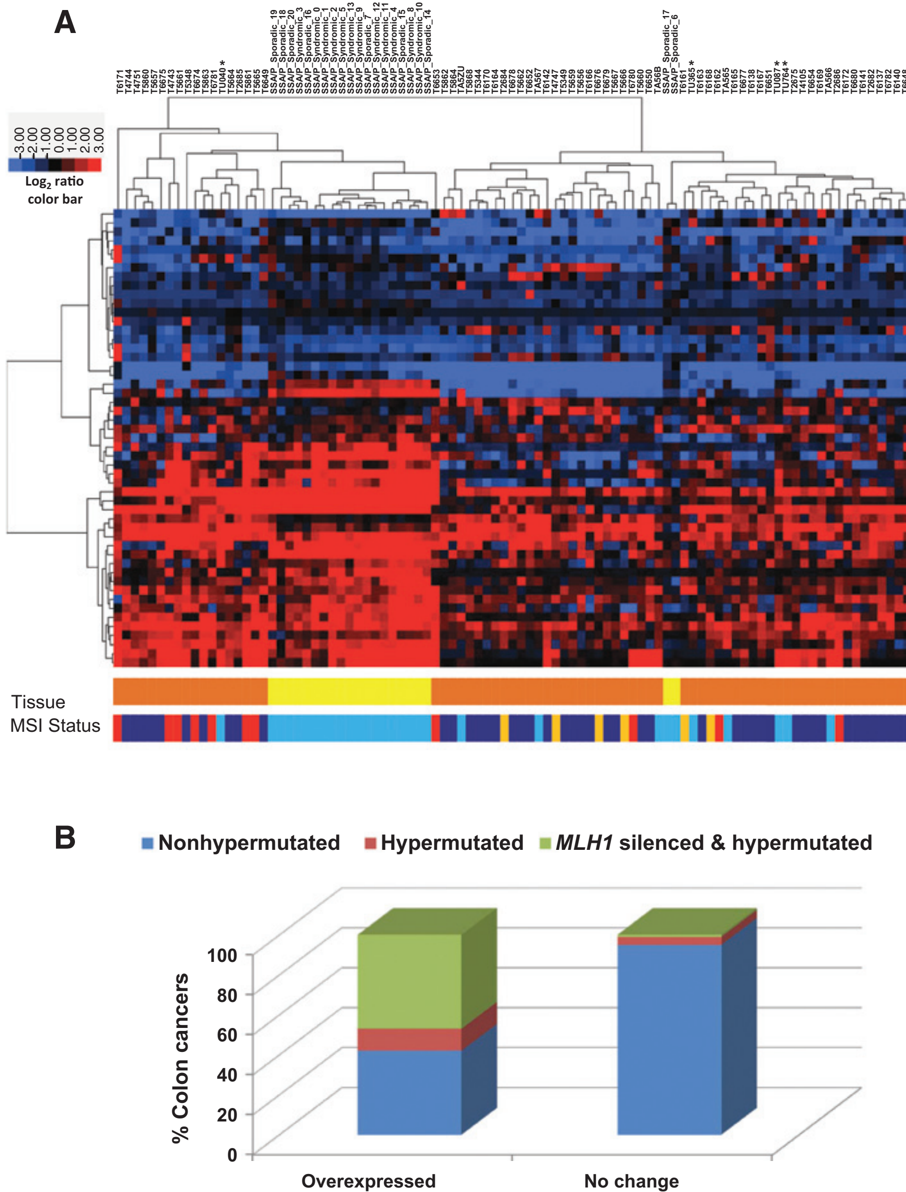

Figure 4.
Evaluation of a 51 SSA/P gene signature in colon cancer RNA-Seq datasets from TCGA. A, $\log_2$ ratios comparing individual colon cancers ($n = 72$) and SSA/Ps ($n = 21$) with the mean of 14 uninvolved and 10 control colon samples ($n = 24$) were used for hierarchical clustering. "Tissue" color bar, colon adenocarcinomas (orange) and SSA/Ps (yellow). "MSI status" color bar, microsatellite-stable (MSS) cancers (dark blue), MSI-H cancers (red), and MSI low cancers (light orange). SSA/Ps and colon cancers not evaluated for MSI (light blue). B, percentage of TCGA colon cancers showing overexpression of *FSCN1*, *ZIC2*, *ZIC5*, *CRYBA2*, *MUC6*, *TRNP1*, and/or *SEMG1* described in Table 1. A total of 195 colon cancers with RNA expression and *MLH1* methylation data in the TCGA database were evaluated using the cBioPortal for Cancer Genomics. *University of Utah Cancer Samples.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

ability to identify a gene signature more closely associated with sporadic colon cancer developing from the serrated pathway.

Three genes (*FSCN1*, *TRNP1*, and *ZIC2*) of our 7-gene panel were previously identified to be overexpressed in *BRAF*-positive colon cancers in a European patient cohort (34). These genes were part of a 64-gene expression classifier for *BRAF*-positive colon cancers with poor prognosis. Another study classifying colon cancers into four consensus molecular subtypes with subtype 1 (CMS1) consisting of microsatellite unstable, CIMP-H, and *BRAF*-positive tumors identified one of our 7-gene panel (*ZIC2*) as a marker of serrated cancers (35, 36). ZIC proteins play a role in regulating the sonic hedgehog and Wnt/B–catenin signaling pathways (37, 38). ZIC2 expression has been associated with multiple cancers, including brain, ovarian, and cervical cancer (39, 40). FSCN1 is an actin-binding protein frequently overexpressed in a variety of cancers, including colon cancer, and predicts poor prognosis (41).FSCN1 is also highly expressed in serrated colon cancers (42). TMF-regulated nuclear protein (TRNP1) is a nuclear protein that plays a role in mammalian brain cortex development (43). The significance of *TRNP1* overexpression in colon cancer remains unknown. Our study reinforces the importance of these genes in serrated colon cancers, providing the first evidence that these mRNA changes occur early in the cancer process in preneoplastic serrated lesions (SSA/Ps).

Other genes described in our 7-gene panel may also participate in colon cancer progression. MUC6 is a gastric mucin protein, shown to have increased expression in SSA/Ps compared with HPs (44). Increased expression of MUC6 has been documented in hypermethylated colon cancers, suggesting its possible role in serrated pathway (45). Data lack about the role of *SEMG1* and *CRYBA2* in colon cancer. SEMG1 is a seminal vesicle protein that has been studied as a biomarker for the detection of prostate

Table 1. Frequency of increased mRNA expression in SSA/P signature genes in 30 hypermutated and 165 nonhypermutated colon cancers from TCGA

Gene symbol	Gene description	Hypermutated CC Incidence (%)	Nonhypermutated CC Incidence (%)	Fisher exact *P*
FSCN1	Fascin actin-binding protein 1	9 (30)	3 (2)	<0.001
ZIC5	Zic family member 5	7 (23)	5 (3)	<0.001
CRYBA2	Crystallin, β A2	5 (17)	0 (0)	<0.001
SEMG1	Semenogelin	4 (13)	0 (0)	<0.001
ZIC2	Zic family member 2	6 (20)	4 (2)	0.001
TRNP1	TMF1-regulated nuclear protein 1	6 (20)	5 (3)	0.002
MUC6	Mucin 6	4 (13)	1 (1)	0.002
FOSL1	FOS-like antigen 1	3 (10)	1 (1)	0.012
ALDHIL1	Aldehyde dehydrogenase 1 family member L1	5 (17)	7 (4)	0.022
KLK10	Kallikrein-related peptidase 10	3 (10)	4 (2)	0.075
SLC18A1	Solute carrier family 18, member 1	3 (10)	4 (2)	0.075
VNN1	Vanin 1	3 (10)	4 (2)	0.075
MUC17	Mucin 17	3 (10)	13 (8)	0.717
ANXA10	Annexin A10	1 (3)	2 (1)	0.396
CLDN1	Claudin 1	1 (3)	11 (7)	0.696

NOTE: Incidence of increased mRNA in 195 colon cancers (30 hypermutated and 165 nonhypermutated) was obtained using TCGA data available in the cBioPortal for Cancer Genomics, Memorial Sloan-Kettering Cancer Center. Table lists 13 of 51 signature genes that show frequent (≥10%) increased mRNA expression in hypermutated colon cancers. Incidence of increased mRNA expression is also shown for two previously developed SSA/P gene markers, Annexin A10 (*ANXA10*) and claudin 1 (*CLDN1*). Changes in mRNA expression were obtained by comparing normalized read counts (RPKM) for each gene across colon cancers diploid for each gene. Statistical significant differences between incidence of increased mRNA expression between hypermutated and nonhypermutated were determined using a Fisher exact test. Nine genes showed statistically significant increased incidence of mRNA overexpression in hypermutated colon cancers.
Abbreviation: CC, colon cancer.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

cancer (46). *CRYBA2* belongs to β/γ-crystallin family of genes and is found to be hypermethylated in CIMP-H neuroblastoma tumors (47). Further mechanistic studies will be needed to understand the functions of these key genes in the serrated pathway.

Table 2. Sensitivity and specificity of a 7-gene panel in identifying *BRAF*-mutant, CIMP-H, and/or *MLH1*-silenced colon cancers from TCGA

A. Individual genes[a]		
Gene	**Sensitivity**	**Specificity**
ZIC5	0.677	0.887
ZIC2	0.548	0.854
FSCN1	0.516	0.947
SEMG1	0.484	0.960
TRNP1	0.484	0.947
CRYBA2	0.419	0.960
MUC6	0.258	0.987
ANXA10	0.194	0.974
CLDN1	0.065	0.881
B. Seven-gene panel[b]		
Minimum # genes positive	**Sensitivity**	**Specificity**
1	0.935	0.722
2	0.839	0.874
3	0.613	0.960
4	0.419	0.987
5	0.290	1.000
6	0.194	1.000
7	0.097	1.000

NOTE: Normalized RNA-Seq gene expression data (RPKM) for each of the 7-gene panel was downloaded from the cBioPortal for Cancer Genomics using the CGDS-R package http://www.cbioportal.org/cgds_r.jsp. A total of 186 TCGA colon cancers had mRNA expression, *BRAF* mutation, methylation subtype, and *MLH1* methylation data available. Thirty-one of 186 colon cancers (17%) were *BRAF* mutated, CIMP-H, and/or *MLH1* silenced. The majority of these cancers (20/31; 64%) had two or more of these DNA alterations, highly suggestive of colon cancers developing via the serrated pathway.
[a]The sensitivity and specificity of each of our 7-gene panel, and two previously described SSA/P gene markers (*ANXA10, CLDN1*), in identifying *BRAF*-mutant, CIMP-H, and/or *MLH1*-silenced colon cancers.
[b]The sensitivity and specificity of one or more genes from our 7-gene panel showing a ≥2-fold increased expression in serrated pathway cancers compared with the average of all colon cancers.

A significant number of the genes that were differently expressed in the uninvolved colonic mucosa of patients with syndromic (SPS) and sporadic SSA/Ps, relative to normal colon (patients with no polyps), overlapped and suggest a field effect may be present in the colonic mucosa of patients with SSA/Ps. These genes were different from those found common to syndromic and sporadic SSA/Ps and had smaller fold changes relative to controls. A "field cancerization" effect has been reported in studies of sporadic colon cancer (48, 49). There are also limited studies investigating possible field effects in patients with colon polyps, particularly SSA/Ps (50). Our data raise important questions regarding the origin of such changes. The question of predictive value of field effect will require studies with larger number of patients, which are underway at this time.

MSI, CIMP, and the inactivation of *MLH1* and *BRAF* mutations have all been implicated as underlying events in the serrated pathway to colon cancer (14–18, 51). A recent study showed *MLH1* silencing in a subgroup of hypermutated colon cancers that had increased *BRAF* and decreased *APC* and *KRAS* mutations. The authors concluded that *MLH1* silencing occurred through a different pathway, suggestive of the serrated pathway (52). However, not all SSA/Ps have these changes, and it remains uncertain whether they are absolute requirements for progression to cancer. A recent large serrated polyp study only identified *MLH1* methylation in 11% of SSA/Ps (53). We report a new set of 51 genes that are differently expressed in most SSA/Ps and sporadic MSI-high cancers in the TCGA cancer database. A smaller 7-gene panel identified *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers with both high sensitivity and specificity. Our findings provide novel molecular markers for SSA/Ps that may play a role in the development of serrated colon cancers.

Limitations of our study include a small sample size in each individual patient cohort ($n = 9–12$). This is, in part, due to colon biopsies being collected prospectively and the low prevalence of

sporadic SSA/Ps and the serrated polyposis syndrome in the general population. Even with this limitation, this is the largest RNA-Seq study performed to characterize the transcriptome of SSA/Ps. Finally, our gene panel was not validated in a separate RNA-Seq study of serrated polyps because these datasets are not publicly available. However, our gene signature did accurately classify SSA/Ps from MVHPs using expression data from a previous microarray study. Future validation studies are currently being designed and are beyond the scope of this study.

In summary, this report provides a comprehensive gene expression comparison of SSA/Ps with HPs, which share many histopathologic similarities but differ markedly in the risk of progression to colon cancer. Despite many similarities in gene expression in SSA/Ps and HPs, both sporadic and syndromic SSA/Ps have a unique gene signature with a number of highly differentially expressed genes of interest relative to oncogenesis. The identification of a set of novel genes uniquely differentially expressed in SSA/Ps and *BRAF*-mutant, CIMP-H, and *MLH1*-silenced colon cancers provides additional leads for further understanding the molecular pathways leading to cancer progression via the serrated pathway. This may lead to the development of a gene panel that can be used in clinical practice to stratify patients with increased colon cancer risk from serrated polyps. This could be especially helpful in identifying patients with serrated polyposis syndrome in whom no currently recognized genetic mutation has been identified.

Disclosure of Potential Conflicts of Interest

R.W. Burt has received speakers bureau honoraria from Myriad Genetics and Thetis Pharma. D.A. Delker and P. Kanth reports having ownership interest (including patents). No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: P. Kanth, M. P. Bronner, R.W. Burt, C.H. Hagedorn, D.A. Delker
Development of methodology: P. Kanth, M. P. Bronner, D.A. Delker
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Kanth, M. P. Bronner, R.W. Burt, D.W. Neklason, D.A. Delker
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Kanth, M. P. Bronner, K.M. Boucher, C.H. Hagedorn, D.A. Delker
Writing, review, and/or revision of the manuscript: P. Kanth, M. P. Bronner, K.M. Boucher, R.W. Burt, D.W. Neklason, C.H. Hagedorn, D.A. Delker
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Kanth, M. P. Bronner, R.W. Burt, D.A. Delker
Study supervision: M. P. Bronner, D.A. Delker

Acknowledgments

The authors thank Kathleen Boynton and Michelle Done for their assistance in sample collection and Mark Hazel for qPCR validation experiments.

Grant Support

This study was supported by a pilot clinical research award from American College of Gastroenterology (to P. Kanth), University of Utah Personalized Medicine Program Seed Grant (to C.H. Hagedorn), NIH grants CA176130 and CA148068 (to C.H. Hagedorn) and CA073992 and CA146329 (to R.W. Burt). This study was also supported by Cancer Center Support Grant P30-CA42014 and National Center for Advancing Translational Sciences of the NIH Award 1ULTR001067 and Huntsman Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 15, 2015; revised March 3, 2016; accepted March 22, 2016; published OnlineFirst March 29, 2016.

References

1. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. 2010. Available from: http://www.cdc.gov/.
2. Burgess NG, Pellise M, Nanda KS, Hourigan LF, Zanati SA, Brown GJ, et al. Clinical and endoscopic predictors of cytological dysplasia or cancer in a prospective multicentre study of large sessile serrated adenomas/polyps. Gut 2016;65:437–46.
3. Lieberman DA, Weiss DG, Bond JH, Ahnen DJ, Garewal H, Chejfec G. Use of colonoscopy to screen asymptomatic adults for colorectal cancer. Veterans Affairs Cooperative Study Group 380. N Engl J Med 2000; 343:162–8.
4. Kahi CJ, Hewett DG, Norton DL, Eckert GJ, Rex DK. Prevalence and variable detection of proximal colon serrated polyps during screening colonoscopy. Clin Gastroenterol Hepatol 2011;9:42–6.
5. Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumors of the digestive system. Lyon: IARC; 2010.
6. Torlakovic E, Skovlund E, Snover DC, Torlakovic G, Nesland JM. Morphologic reappraisal of serrated colorectal polyps. Am J Surg Pathol 2003; 27:65–81.
7. Wong NA, Hunt LP, Novelli MR, Shepherd NA, Warren BF. Observer agreement in the diagnosis of serrated polyps of the large bowel. Histopathology 2009;55:63–6.
8. Khalid O, Radaideh S, Cummings OW, O'Brien MJ, Goldblum JR, Rex DK. Reinterpretation of histology of proximal colon polyps called hyperplastic in 2001. World J Gastroenterol 2009;15:3767–70.
9. Jasperson KW, Kanth P, Kirchhoff AC, Huismann D, Gammon A, Kohlmann W, et al. Serrated polyposis: colonic phenotype, extracolonic features, and familial risk in a large cohort. Dis Colon Rectum 2013;56: 1211–6.
10. Rashid A, Houlihan PS, Booker S, Petersen GM, Giardiello FM, Hamilton SR. Phenotypic and molecular characteristics of hyperplastic polyposis. Gastroenterology 2000;119:323–32.
11. Boparai KS, Mathus-Vliegen EM, Koornstra JJ, Nagengast FM, van Leerdam M, van Noesel CJ, et al. Increased colorectal cancer risk during follow-up in patients with hyperplastic polyposis syndrome: a multicentre cohort study. Gut 2010;59:1094–100.
12. Holme O, Bretthauer M, Eide TJ, Loberg EM, Grzyb K, Loberg M, et al. Long-term risk of colorectal cancer in individuals with serrated polyps. Gut 2015;64:929–36.
13. Mäkinen M.J. Colorectal serrated adenocarcinoma. Histopathology 2007; 50:131–50.
14. O'Brien MJ. Hyperplastic and serrated polyps of the colorectum. Gastroenterol Clin North Am 2007;36:947–68.
15. O'Brien MJ, Zhao Q, Yang S. Colorectal serrated pathway cancers and precursors. Histopathology 2015;66:49–65.
16. Iino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y, et al. DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? J Clin Pathol 1999;52:5–9.
17. Guarinos C, Sánchez-Fortún C, Rodríguez-Soler M, Perez-Carbonell L, Egoavil C, Juarez M, et al. Clinical subtypes and molecular characteristics of serrated polyposis syndrome. Clin Gastroenterol Hepatol 2013;11:705–11.
18. Samowitz WS, Albertsen H, Herrick J, Levin TR, Sweeney C, Murtaugh MA, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. Gastroenterology 2005;129: 837–45.
19. Gonzalo DH, Lai KK, Shadrach B, Goldblum JR, Bennett AE, Downs-Kelly E, et al. Gene expression profiling of serrated polyps identifies annexin A10 as a marker of a sessile serrated adenoma/polyp. J Pathol 2013;230:420–9.
20. Caruso M, Moore J, Goodall GJ, Thomas M, Phillis S, Tyskin A, et al. Overexpression of cathepsin E and trefoil factor 1 in sessile serrated adenomas of the colorectum identified by gene expression analysis. Virchows Arch 2009;454:291–302.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024

21. Delker DA, McGettigan BM, Kanth P, Pop S, Neklason DW, Bronner MP, et al. RNA sequencing of sessile serrated colon polyps identifies differentially expressed genes and immunohistochemical markers. PLoS One 2014;9:e88367.
22. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR, et al. United States Multi-Society Task Force on Colorectal Cancer. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. Gastroenterology 2012;143:844–57.
23. Folkers ME, Delker DA, Maxwell CI, Nelson CA, Schwartz JJ, Nix DA, et al. ENCODE tiling array analysis identifies differentially expressed annotated and novel 5′ capped RNAs in hepatitis C infected liver. PLoS One 2011;6:e14697.
24. Papic N, Maxwell CI, Delker DA, Liu S, Heale BS, Hagedorn CH. RNA-sequencing analysis of 5′ capped RNAs identifies many new differentially expressed genes in acute hepatitis C virus infection. Viruses 2012;4:581–612.
25. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. Genome Biol 2014;15:550.
26. Comprehensive molecular characterization of human colon and rectal cancer. Cancer Genome Atlas Network. Nature 2012;487:330–7.
27. National Institutes of Health. TCGA data portal. Available from: https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp.
28. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signali 2013;6:pl1.
29. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.
30. Ciriello G, Cerami E, Sander C, Schultz N. Mutual exclusivity analysis identifies oncogenic network modules. Genome Res 2012;22:398–406.
31. Caruso M1, Fung KY2, Moore J3, Brierley GV, Cosgrove LJ, Thomas M, et al. Claudin-1 expression is elevated in colorectal cancer precursor lesions harboring the BRAF V600E. Transl Oncol 2014;7:456–63.
32. DiSario JA, Foutch PG, Mai HD, Pardy K, Manne RK. Prevalence and malignant potential of colorectal polyps in asymptomatic, average-risk men. Am J Gastroenterol 1991;86:941–5.
33. Weston AP, Campbell DR. Diminutive colonic polyps: histopathology, spatial distribution, concomitant significant lesions, and treatment complications. Am J Gastroenterol 1995;90:24–8.
34. Popovici V, Budinska E, Tejpar S, Weinrich S, Estrella H, Hodgson G, et al. Identification of a poor-prognosis BRAF-mutant-like population of patients with colon cancer. J Clin Oncol 2012;30:1288–95.
35. Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.
36. De Sousa EMelo F, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 2013;19:614–8.
37. Merzdorf CS. Emerging roles for zic genes in early development. Dev Dyn 2007;236:922–40.
38. Sanek NA, Taylor AA, Nyholm MK, Grinblat Y. Zebrafish zic2a patterns the forebrain through modulation of Hedgehog-activated gene expression. Development 2009;136:3791–800.
39. Marchini S, Poynor E, Barakat RR, Clivio L, Cinquini M, Fruscio R, et al. The zinc finger gene ZIC2 has features of an oncogene and its overexpression correlates strongly with the clinical course of epithelial ovarian cancer. Clin Cancer Res 2012;18:4313–24.
40. Chan DW, Liu VW, Leung LY, Yao KM, Chan KK, Cheung AN, et al. Zic2 synergistically enhances Hedgehog signalling through nuclear retention of Gli1 in cervical cancer cells. J Pathol 2011;225:525–34.
41. Ma Y, Machesky LM. Fascin1 in carcinomas: Its regulation and prognostic value. Int J Cancer 2015;137:2534–44.
42. Conesa-Zamora P, García-Solano J, García-García F, Turpin Mdel C, Trujillo-Santos J, Torres-Moreno D, et al. Expression profiling shows differential molecular pathways and provides potential new diagnostic biomarkers for colorectal serrated adenocarcinoma. Int J Cancer 2013;132:297–307.
43. Stahl R, Walcher T, De Juan Romero C, Pilz GA, Cappello S, Irmler M, et al. Trnp1 regulates expansion and folding of the mammalian cerebral cortex by control of radial glial fate. Cell 2013;153:535–49.
44. Owens SR, Chiosea SI, Kuan SF. Selective expression of gastric mucin MUC6 in colonic sessile serrated adenoma but not in hyperplastic polyp aids in morphological diagnosis of serrated polyps. Mod Pathol 2008;21:660–9.
45. Walsh MD, Clendenning M, Williamson E, Pearson SA, Walters RJ, Nagler B, et al. Expression of MUC2, MUC5AC, MUC5B, and MUC6 mucins in colorectal cancers and their association with the CpG island methylator phenotype. Mod Pathol 2013;26:1642–56.
46. Neuhaus J, Schiffer E, von Wilcke P, Bauer HW, Leung H, Siwy J, et al. Seminal plasma as a source of prostate cancer peptide biomarker candidates for detection of indolent and advanced disease. PLoS One 2013;8:e67514.
47. Abe M, Watanabe N, McDonell N, Takato T, Ohira M, Nakagawara A, et al. Identification of genes targeted by CpG island methylator phenotype in neuroblastomas, and their possible integrative involvement in poor prognosis. Oncology 2008;74:50–60.
48. Hawthorn L, Lan L, Mojica W. Evidence for field effect cancerization in colorectal cancer. Genomics 2014;103:211–21.
49. Lochhead P, Chan AT, Nishihara R, Fuchs CS, Beck AH, Giovannucci E, et al. Etiologic field effect: reappraisal of the field effect concept in cancer predisposition and progression. Mod Pathol 2015;28:14–29.
50. Chen LC, Hao CY, Chiu YS, Wong P, Melnick JS, Brotman M, et al. Alteration of gene expression in normal-appearing colon mucosa of APC(min) mice and human cancer patients. Cancer Res 2004;64:3694–700.
51. Huang CS, Farraye FA, Yang S, O'Brien MJ. The clinical significance of serrated polyps. Am J Gastroenterol 2011;106:229–40.
52. Donehower LA, Creighton CJ, Schultz N, Shinbrot E, Chang K, Gunaratne PH, et al. MLH1-silenced and non-silenced subgroups of hypermutated colorectal carcinomas have distinct mutational landscapes. J Pathol 2013;229:99–110.
53. Burnett-Hartman AN, Newcomb PA, Potter JD, Passarelli MN, Phipps AI, Wurscher MA, et al. Genomic aberrations occurring in subsets of serrated colorectal lesions but not conventional adenomas. Cancer Res 2013;73:2863–72.

Downloaded from http://aacrjournals.org/cancerpreventionresearch/article-pdf/9/6/456/1936408/456.pdf by guest on 12 April 2024