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Influence of target gene mutations on survival, stage and histology in sporadic microsatellite unstable colon cancers

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Abstract

High-frequency microsatellite unstable (MSI-H) colon tumors develop as a consequence of mutations at repetitive sequences in target genes. *TGFBR2* and *ACVR2*, encoding TGFb superfamily receptors, and the proapoptotic gene *BAX* are frequent targets for frameshift mutation. We analyzed the effect of these mutations on survival and histology in 2 separate cohorts. Forty-eight MSI-H Dukes B2 colon tumors from a cohort of 172 patients had mutations in *TGFBR2*, *BAX* and *ACVR2* correlated with patient survival. Further, 54 population-based MSI-H colon cancers of all stages from a cohort of 503 patients had mutations correlated with tumor stage, grade and size. Of 44 amplifiable MSI-H Dukes B2 tumors, 70% harbored *TGFBR2*, 63% *BAX* and only 4.5% *ACVR2* mutations. While mutation alone did not influence survival, concomitant mutation of *TGFBR2* and *BAX* was associated with an improved prognosis in Dukes B2 patients ($p = 0.05$). *ACVR2* mutations were more frequent in the second, population-based cohort (stage II: 32.5%, $p < 0.05$). While no target gene mutation correlated with stage in this cohort, poor histological grade and large tumor volume were associated with mutant *ACVR2*, but not *TGFBR2* or *BAX* mutations, and likely accounts for the lower prevalence of *ACVR2* mutations in the first, well-differentiated Dukes B2 cohort. Because target gene mutations did not correlate with stage, they likely occur early in the pathogenesis of MSI-H cancers. Mutations in *TGFBR2* and *BAX* may improve survival in MSI-H Dukes B2 patients, and mutations of *ACVR2* may augment histological changes consistent with poor tumor grade that is characteristic of MSI-H colon cancers, and increase tumor size.

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Keywords

activin; DNA mismatch repair; transforming growth factor β; *BAX*; microsatellite instability

Microsatellite unstable colon cancers are thought to develop genetically when key genes containing coding microsatellites become frameshifted, inactivating the gene's expressed product. *TGFBR2* encodes a receptor for TGFβ1, which seems to function as a growth suppressor during colon tumor development, but later may enhance metastases. *TGFBR2* contains an A_{10} microsatellite that is mutated in 60–90% of MSI-H colon cancers.[1,2,3] *BAX* encodes a proapoptotic protein important in triggering cell death and thus is a tumor suppressor. Its G_8 microsatellite is mutated in 40–60% of MSI-H colon cancers.[4,5] *ACVR2* encodes a specific receptor for activin, a TGFβ superfamily member. Its role in colon cancer development is not fully clear, but it is likely to act as a tumor suppressor. *ACVR2* contains 2 coding A_8 microsatellites, of which the exon 10 microsatellite is frequently mutated in colon cancer.[6,7]

Unlike chromosomal unstable colon cancers in which an accepted genetic pattern has been well established for colon cancer progression that matches histological correlates, an apparent pattern in MSI-H colon cancer is less clear. In MSI-H colon cancers, mutations in *TGFBR2* from tumors of stage III colon cancer patients is associated with an improved survival.[2] The onset of *TGFBR2* mutations likely occur in high-grade dysplasia at the interface between a benign adenoma and the development of carcioma.[1] *BAX* may also become mutated at this interface,[5] which may affect responses to therapy or chemoprevention.[8] There are no data in this regard for *ACVR2* in MSI-H colon cancers, nor is there any data on histological correlates with *ACVR2* mutations.

The importance of understanding the genetic changes in MSI-H colon cancers lies in the difference seen in survival and response to therapy. For instance, several groups have shown that patients with MSI-H colon cancer have an overall better survival than patients with non-MSI-H tumors.[9,10] Additionally, patients with MSI-H colon cancer do not respond to 5-FU with increased survival like their non-MSI-H counterparts.[11,12] Distinguishing features of MSI-H tumors include: poorer grade, mucinous histology, a surrounding lymphocytic infiltrate and location proximal to the splenic flexure of the colon.[13]

Here, we examined 2 cohorts to assess the influence of these target gene mutations in MSI-H colon cancer. Specifically, we determined the survival outcome in node-negative MSI-H colon cancers with *TGFBR2*, *BAX* and *ACVR2* mutations. We also assessed mutational status in a more advanced cohort and correlated target gene mutations with stage, and tumor grade and size.

Material and methods

Patient selection and data collection

A total cohort of 172 patients with stage II (Astler-Collier-Dukes B2) colon cancers from the U.S. (*N* = 84, which includes 70 from our previous publication[14] plus an additional 14 patients from the University of California, San Diego Medical Center) and Switzerland (*N* =

88) were previously collected between 1984 and 1989.[14,15] A second cohort of 503 patients with colon tumors were prospectively collected as part of the North Carolina Colon Cancer Study (NCCCS), a population-based, case–control study comprising 503 patients.[16] Both studies were performed under IRB approval. The MSI status of most tumors in both cohorts had been previously determined.[7,14–16]

All tumors were formalin-fixed and embedded in paraffin, then sliced into 5 μm sections. A reference hematoxylin and eosin stain was performed on one cut, and on subsequent cuts, the normal and tumor tissue were determined and marked for microdissection by a single pathologist (KM), who was blinded to the results of the target gene analysis. Histological grading was scored by the same experienced gastrointestinal pathologist using previously described criteria for determining poor, moderate and well differentiation.[17]

DNA extraction

DNA was extracted from formalin-fixed, paraffin-embedded tissues of each patient's colon tumors and the surrounding noncancerous tissue as described previously.[7,14] Unstained tissues slices adjacent to the reference H&E stained slide were microdissected, according to areas identified on the reference slide, by using a surgical scalpel blade, and areas of cancer microdissected had >90% tumor cells. The dissected specimen was deparaffinized in a microfuge tube with xylene, and the DNA was purified with ethanol and GeneReleaser (Bio Ventures, Murfeesboro, TN), according to the manufacturer's recommendations. Subsequently, the samples were treated with 200 ug/ml of proteinase K (Sigma, St. Louis, MO) and incubated at 55°C for 5 hr. Proteinase K was destroyed by heating the sample to 95°C for 15 min, and the samples were immediately iced and stored for polymerase chain reaction (PCR) analysis.

Microsatellite analysis

MSI status was determined in all cancers using the National Cancer Institute-recommended panel of 5 microsatellite markers (BAT25, BAT26, D5S346, D2S123 and D17S250)[18] to classify the tumor as MSI-high (associated with inactivation of DNA MMR) or microsatellite stable (MSS), which is not associated with DNA MMR inactivation.

Primers were radiolabeled with 0.1 μCi (1 Ci = 37 GBq) of [γ-^{32}P]dATP (DuPont/NEN). PCR products were separated on 6% polyacrylamide gels containing 6 M urea, followed by autoradiography. Mutations were determined by a change in the electrophoretic mobility of the PCR products.

MSI-H tumors were defined as ≥2 of 5 markers with novel alleles compared to matched nontumor DNA, whereas MSS tumors had 0 of 5 markers with novel alleles. Tumors with 1/5 markers positive were defined as MSI-low and were included with the MSS group as non-MSI tumors. MSI-low tumors do not have MMR gene loss or inactivation.[18]

Amplification of the polyadenine tracts of target genes

Specific primers were designed to amplify the polyadenine tracts in exon 3 and exon 10 of *ACVR2* (exon 3, forward 5′-TCTGCTTATTTATAGGACTGATTGTG-3′ and reverse 5′-

CGCTGTGTGACTTCCATCTC-3′; exon 10, forward 5′-GTTGCCATTTGAGGAGGAAA-3′ and reverse 5′-CCTCTGAAAAGTGTTTTATTGGAA-3′) as well as *TGFBR2* (forward 5′-CTTTATTCTGGAAGATGCTG-3′ and reverse 5′-GAAGAAAGTCTCACCAGGC-3′) and *BAX* (forward 5′-ATCCAGGATCGAGCAGGGCG-3′ and reverse 5′-ACTCGCTCAGCTTCTTGGTG-3′). One primer from each set was radiolabeled with ^{32}P, and DNA was amplified in a thermocycler (MJ Research, Waltham, MA) in a reaction containing 1 μM of each primer, 1× reaction buffer, 100 ng DNA template, 200 μM deoxynucleotides, 1.5 μM magnesium chloride and 2.5 U Taq polymerase. PCR was carried out over 29 cycles of 94, 54 and 72°C of 1 min each, preceded by a 3 min denaturing step at 94°C and followed by a 10 min extension step at 72°C. After PCR, the product bands were analyzed on a 6% polyacrylamide gel and viewed with a phosphorimager (Molecular Dynamics, Sunnyvale, CA) for band shifts comparing the tumor DNA to the paired normal DNA. Each reaction and electrophoresis was repeated at least twice. In most of the cases, direct DNA sequencing using an automated DNA Analyzer (Perkin Elmer) was used to confirm mutations identified on the gels.

Statistical analysis

Statistical analyses were done by the UCSD Moores Comprehensive Cancer Center Biostatistic Shared Resource utilizing SAS software. The following variables were assessed: age, gender, location of the tumor within the colon, as well as stage, follow-up time and vital status (alive or dead) if available. The location of the tumor was classified as right if the tumor was at or proximal to the splenic flexure. Left-sided tumors were classified distal to the splenic flexure. Disease stage was classified at surgery. Histological variables included the grade of the tumor (well, moderate or poorly differentiated). Statistical analyses of these descriptive values were as follows: for continuous variables, the *t*-test; for categorical values, Fisher's Exact Test; for pathological values, χ^2 analysis.

Five-year survival analyses were done after classifying MSI-H tumors from patients and whether their tumors had the absence or presence of target gene mutations. To examine differences in survival rates, a Cox proportional hazard function was used for both univariate and multivariate analyses. Univariate and multivariate survival distributions were compared with the use of the logrank test. Multivariate analysis was only used to examine the combined *BAX* and *TGFBR2* mutations (controlled for tumor location) on survival. Significance for all statistics, the likelihood of a difference between groups, was recorded if the *p* value was 0.05 or less.

Results

Target gene mutations and survival in MSI-H Dukes B2 tumors

Table I depicts the clinical data from our Dukes B2 cohort, separated by MSI status. We found no difference between gender ($p = 0.88$), follow-up (60.3 months for MSI-H *vs.* 58.9 months for MSS, $p > 0.05$) or age at diagnosis ($p = 0.99$) in patients with MSI-H *versus* MSS tumors in this Dukes B2 cohort. Patients with MSI-H tumors had a lower overall mortality than patients with MSS tumors, but this did not reach statistical significance (14%

vs. 24%, $p = 0.20$). MSI-H Dukes B2 tumors were significantly more likely to be located proximally in the colon (73% *vs.* 31%, $p < 0.001$). Overall, tumor grade was well to moderately-differentiated in 87% of MSI-H tumors, not dissimilar to the MSS tumors within the same cohort (93%) ($p = 0.48$) (Table I).

From the 172 Dukes B2 colon tumors analyzed, 48 (28%) were MSI-H, consistent with previous reports of higher prevalences of MSI-H within earlier-staged tumors.[2,19] Forty-four tumors had DNA that amplified at all 3 target gene loci. Of those 44, 31 (70%) harbored *TGFBR2* mutations, 28 (63%) harbored *BAX* mutations, and only 2 (4.5%) had *ACVR2* mutations. Fifty-two percent of all MSI-H tumors had mutations in both *BAX* and *TGFBR2* and the 2 tumors with *ACVR2* mutations also had mutations in *TGFBR2* and *BAX*.

Contrary to what has been reported in stage III patients,[2] we found no difference in survival in our stage II patients with MSI-H tumors comparing those with and without *TGFBR2* mutations (Table II). Similarly, there was no difference with *BAX* mutations and survival (Table II). However, patients with both *TGFBR2* and *BAX* mutations had a survival benefit ($p = 0.05$), when compared to patients with 1 or no mutations present. When controlling for tumor location utilizing multivariate analysis, *TGFBR2* and *BAX* mutations together trended as an independent predictive factor for survival ($p = 0.09$). Comparing the various combinations of the *TGFBR2* (mutated and wild type) and *BAX* (mutated and wild type) gene status, we found no statistical differences between the combination of groups.

Target gene mutations occur independent of tumor stage

Because only 2 *ACVR2* mutations were found in our Dukes B2 cohort, we analyzed a second cohort from North Carolina, which we had previously examined for *ACVR2* mutations, to determine whether patient staging might explain the differences. This cohort, which lacked survival data, was similar to our Dukes B2 cohort, in that there was no difference in gender (although there was a trend toward more females in the MSI-H group, $p = 0.06$) or age at presentation ($p = 0.9$), and MSI-H tumors were more likely to be located in the proximal colon ($p < 0.001$) (Table III). However, this cohort is different from the Dukes B2 cohort, in that MSI-H tumors had a significantly higher proportion of poorly-differentiated tumors compared to MSS tumors. We found a higher number of *ACVR2* mutations in stage II patients in this cohort *versus* the Dukes B2 cohort (32.5% *vs.* 4.5%, $p < 0.05$). Overall, the NCCCS cohort had 54/503 (11%) patients with MSI-H tumors, and 45/54 (83%) had *ACVR2* mutated.[7] Forty-one patients with MSI-H tumors from this cohort with known stage (2% A, 36% B, 57% C and 5% D) were analyzed as early (Dukes A + B) or advanced (Dukes C + D) tumors with target gene mutational status. None of these assessed target genes independently correlated with tumor stage, nor did the combined presence of *ACVR2*, *TGFBR2* and *BAX* mutations (Table IV).

ACVR2, but not TGFBR2 or BAX mutations, correlate with histologic grade and size of tumor

Forty-seven of the MSI-H tumors from our NCCCS cohort had both grade and *ACVR2* status available. Of 20 well- and moderately-differentiated tumors, 6 (30%) had wild-type *ACVR2*, while only 1 of 27 (3%) poorly differentiated tumors was *ACVR2* wild type ($p <$

0.01) (Table V), whereas there was no significant difference in tumor grade with or without *TGFBR2* or *BAX* mutations (Table V).

Of the 54 MSI-H tumors with *ACVR2* data, 28 tumors had 3D size data available: tumors containing wild-type *ACVR2* had a mean volume of 35 cm^3 (median 13 cm^3), and tumors containing mutant *ACVR2* had a mean volume of 99 cm^3 (median 47 cm^3) ($p < 0.05$) (Table V). We found no statistical difference in size for tumors with and without *TGFBR2* or *BAX* mutations (Table V).

Discussion

Microsatellite unstable colon cancers develop and progress genetically as a consequence of frameshift mutations in target genes. In this study, we found that: (*i*) although *ACVR2* mutations tended to be less in stage II cancers, than in stage III and IV cancers, *ACVR2* mutations were not significantly associated with stage (nor were *TGFBR2* and *BAX* mutations), (*ii*) *ACVR2*, but not *TGFBR2* or *BAX* mutations, correlated with poorer tumor differentiation, (*iii*) *ACVR2*, but not *TGFBR2* or *BAX* mutations, correlated with larger tumors, and (*iv*) patients with stage II colon cancer had improved survival with mutations in both *TGFBR2* and *BAX*, but not with either alone. There were not enough tumors with *ACVR2* mutations in the Dukes B2 cohort to determine any relationship between the presence of *ACVR2* mutations and patient survival. These findings indicate a role for target genes in the progression of colon cancer, both at the histological stage and in regards to patient survival.

In examining *ACVR2* mutations in our 2 cohorts, we were surprised to find different mutational prevalences among stage II patients. We found that the cohorts are different, in that our Dukes B2 cohort is far better differentiated than our NCCCS cohort (87% *vs.* 42% of MSI-H cancers, respectively) as designated by a single pathologist. Tumors with *ACVR2* mutations are more likely to be poorly differentiated (Table V), which was not the case when examining *TGFBR2* and *BAX* mutations (Table V). We are confident in our detection for frameshift mutations as we have previously shown that all frameshifts detected by gel analysis revealed frameshift mutations upon sequencing, and that majority were biallelic and lead to loss of protein expression.[7] Contrary to that reported in cell lines, compound heterozygotes do not appear to be a major concern in primary colon cancer tissue, at least for detection of *ACVR2* mutations. We also analyzed the exon 3 polyadenine tract of *ACVR2*, and as previously reported,[7] we did not find any mutation of this coding microsatellite in any of the samples. Technical problems with identifying *ACVR2* mutations in MSI-H cancers can be excluded as both MSI-H-specific *TGFBR2* and *BAX* mutations occurred between cohorts, compared to the varied prevalence of *ACVR2* mutations, and were confirmed with repeated assays. Thus, tumor differentiation appears to be one predictor for the presence of *ACVR2* mutations in MSI-H tumors. Although additional differences might contribute toward variation in the frequency of *ACVR2* mutations between the cohorts, this should have little impact on our results above, as both cohorts are analyzed separately for different endpoints (survival in one cohort, and tumor size and differentiation in the other cohort) with no direct comparison made between the cohorts. Last, we found a higher percentage of MSI-H in our Dukes B2 cohort compared to that reported in the literature

grouped for all stages (I–IV) of colon cancer. This is consistent with previous reports of higher prevalences of MSI-H among earlier-staged colon tumors.[2,19] We do acknowledge that the earlier analysis may be limited by the few deaths observed in these Dukes B2 patients (Table II).

We found a significant difference in tumor size comparing MSI-H tumors with and without *AVCVR2*, but not *TGFBR2* or *BAX* mutations. Activin signaling is presumed to be growth suppressive, and abrogation of signaling by a mutant *ACVR2* (and subsequent loss of *ACVR2* expression) would augment growth. Since *ACVR2* mutations segregated with larger tumors, we believe that intact activin signaling may, in part, contribute to the physical size of MSI-H tumors.

Although tumor size and differentiation has no influence on survival,[20] the level of invasion and stage remain the overall best predictors of prognosis.[21] In comparing target gene mutations and survival in our Dukes B2 cohort, we found no survival advantage for patients with *TGFBR2* or *BAX* mutations present. Because this cohort was overall well-differentiated to moderately differentiated, the number of *ACVR2* mutations was too low to make any comparisons. When we combined *TGFBR2* and *BAX* mutations, univariate analysis predicted a survival advantage with this tumor genotype in MSI-H Dukes B2 patients. *TGFBR2* mutations are associated with an increased survival in stage III MSI-H patients.[2] TGFβ signaling loss through *TGFBR2* mutations might confer protection from the "molecular switch" from growth suppression to one of metastasis enhancer,[22] and improve survival after the switch has occurred. In our Dukes B2 cohort, this suppressive to enhancer switch may not be as marked as in stage III patients, as patients with Dukes B2 tumors are discovered prior to the development of metastasis, and thus have improved survival on that basis. That the combination of *BAX* with *TGFBR2* mutations might confer a survival advantage is more curious. Patients with *BAX* mutations within their MSI-H tumors were found to have a diminished survival,[4] although others have published opposite results.[23] Our finding of possible survival synergism between mutated *BAX* and *TGFBR2* in patients with MSI-H Dukes B2 colon cancer may suggest a possible interaction between defective apoptotic pathways and defective TGFβ signaling that may prevent metastatic spread of the tumor.

We found no correlation of target gene mutations and stage of MSI-H tumors. This suggests that *ACVR2*, *TGFBR2* and *BAX* mutations are likely to occur in high-grade dysplasia at the interface of malignancy, which has been clearly described for *TGFBR2* mutations.[1] Likewise, *BAX* mutations in hereditary nonpolyposis colon cancer (HNPCC) tumors were rare in adenomas, but common in cancers, suggesting that mutation occurs during malignant transformation.[5] We suggest here that *ACVR2* mutations also are likely to occur earlier than cancers in our cohort as well. Indeed, this idea is corroborated by a recent publication demonstrating an equal *ACVR2* mutation rate between HNPCC-associated MSI-H adenomas (70.4%) and HNPCC-associated MSI-H carcinomas (71.8%).[24]

In summary, based on our findings examining MSI-H cancers from these 2 cohorts, we suggest that target gene mutations occur prior to malignancy due to the target gene's specific genetic structure in the presence of dysfunctional DNA mismatch repair, but that the

consequence of each target gene mutation may be observed at various times later in cancer pathogenesis. Mutation of *BAX* may contribute to loss of programmed cell death to facilitate malignant transformation, and later by unknown mechanisms further reduce metastases in combination with *TGFBR2* mutation in node-negative cancers. Mutation of *TGFBR2* may initially release the adenoma cells from TGFβ-induced growth suppression, but later protect the host from metastases after some mechanism of functional switching of TGFβ into a metastases enhancer. Mutations of *ACVR2* may contribute to the release of adenoma cells from growth suppression, but also augment histological changes consistent with poor tumor grade that is characteristic of many MSI-H colon cancers, as well as increase local tumor size. Target gene mutational status may influence grade, size and patient survival in MSI-H colon cancers and this is the first report to analyze the clinical impact of *ACVR2* mutations in primary colon cancer specimens.

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References

1. Grady WM, Rajput A, Myeroff L, Liu DF, Kwon K, Willis J, Markowitz S. Mutation of the type II transforming growth factor-beta receptor is coincident with the transformation of human colon adenomas to malignant carcinomas. Cancer Res. 1998; 58:3101–4. [PubMed: 9679977]
2. Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, Benson AB III, Hamilton SR. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001; 344:1196–206. [PubMed: 11309634]
3. Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colon cancer. Cancer Res. 1995; 55:5548–50. [PubMed: 7585632]
4. Ionov Y, Yamamoto H, Krajewski S, Reed JC, Perucho M. Mutational inactivation of the proapoptotic gene BAX confers selective advantage during tumor clonal evolution. Proc Natl Acad Sci USA. 2000; 97:10872–7. [PubMed: 10984511]
5. Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M, Yuasa Y. Proapoptotic gene BAX is frequently mutated in hereditary nonpolyposis colon cancers but not in adenomas. Gastroenterology. 1998; 114:268–74. [PubMed: 9453486]
6. Mori Y, Yin J, Rashid A, Leggett BA, Young J, Simms L, Kuehl PM, Langenberg P, Meltzer SJ, Stine OC. Instabilotyping: comprehensive identification of frameshift mutations caused by coding region microsatellite instability. Cancer Res. 2001; 61:6046–9. [PubMed: 11507051]
7. Jung B, Doctolero RT, Tajima A, Nguyen AK, Keku T, Sandler RS, Carethers JM. Loss of activin receptor type 2 protein expression in microsatellite unstable colon cancers. Gastroenterology. 2004; 126:654–9. [PubMed: 14988818]
8. Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of BAX in the apoptotic response to anticancer agents. Science. 2000; 290:989–92. [PubMed: 11062132]
9. Choi SW, Lee KJ, Bae YA, Min KO, Kwon MS, Kim KM, Rhyu MG. Genetic classification of colon cancer based on chromosomal loss and microsatellite instability predicts survival. Clin Cancer Res. 2002; 8:2311–22. [PubMed: 12114436]
10. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, Redston M, Gallinger S. Tumor microsatellite instability and clinical outcome in young patients with colon cancer. N Engl J Med. 2000; 342:69–77. [PubMed: 10631274]

11. Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K, Boland CR. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colon cancer. Gastroenterology. 2004; 126:394–401. [PubMed: 14762775]
12. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003; 349:247–57. [PubMed: 12867608]
13. Carethers, JM.; Boland, CR. Neoplasia of the gastrointestinal tract. In: Yamada, T.; Alpers, DH.; Kaplowitz, N.; Laine, L.; Owyang, C.; Powell, DW., editors. Textbook of gastroenterology. 4. Philadelphia, PA: Lippincott-Raven; 2003. p. 557-83.
14. Carethers JM, Hawn MT, Greenson JK, Hitchcock CL, Boland CR. Prognostic significance of allelic lost at chromosome 18q21 for stage II colon cancer. Gastroenterology. 1998; 114:1188–95. [PubMed: 9609755]
15. Gervaz P, Cerottini JP, Bouzourene H, Hahnloser D, Doan CL, Benhattar J, Chaubert P, Secic M, Gillet M, Carethers JM. Comparison of microsatellite instability and chromosomal instability in predicting survival of patients with T3N0 colon cancer. Surgery. 2002; 131:190–7. [PubMed: 11854698]
16. Satia JA, Keku T, Galanko JA, Martin C, Doctolero RT, Tajima A, Sandler RS, Carethers JM. Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study. Cancer Epidemiol Biomarkers Prev. 2005; 14:429–43. [PubMed: 15734969]
17. Morson, BC.; Dawson, IP.; Day, DW.; Jass, JR.; Price, AB.; Williams, GT. Malignant epithelial tumors. In: Morson, BC.; Dawson, IP., editors. Gastrointestinal pathology. 3. Oxford, England: Blackwell Scientific; 1990. p. 608-10.
18. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colon cancer. Cancer Res. 1998; 58:5248–57. [PubMed: 9823339]
19. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science. 1993; 260:816–9. [PubMed: 8484122]
20. Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, Matsukuma S, Kanai T, Kurihara H, Ozawa K, Yoshimura K, Bekku S. Risk factors for an adverse outcome in early invasive colon carcinoma. Gastroenterology. 2004; 127:385–94. [PubMed: 15300569]
21. Haggitt RC, Glotzbach RE, Soffer EE, Wruble LD. Prognostic factors in colon carcinomas arising in adenomas: implications for lesions removed by endoscopic polypectomy. Gastroenterology. 1985; 89:328–36. [PubMed: 4007423]
22. Derynck R, Akhurst RJ, Balmain A. TGF-β signaling in tumor suppression and cancer progression. Nat Genet. 2001; 29:117–29. [PubMed: 11586292]
23. Samowitz WS, Curtin K, Neuhausen S, Schaffer D, Slattery ML. Prognostic implications of BAX and TGFBRII mutations in colon cancers with microsatellite instability. Genes Chromosomes Cancer. 2002; 35:368–71. [PubMed: 12378532]
24. Woerner SM, Kloor M, Mueller A, Rueschoff J, Friedrichs N, Buettner R, Buzello M, Kienle P, Knaebel H-P, Kunstmann E, Pagenstecher C, Schackert HK, et al. Microsatellite instability of selective target genes in HNPCC-associated colon adenomas. Oncogene. 2005; 24:2525–35. [PubMed: 15735733]

TABLE I

CHARACTERISTICS OF DUKES B2 COHORT[1]

	Gender	Mean age (years)	Deaths	Location [proximal colon]	Grade [well to moderate]
MSI-H [*N* = 44]	M26 F18	67.1	6/44 (14)	32/44 (73)	39/44 (87)
MSS [*N* = 124]	M69 F55	67.4	30/124 (24)	38/124 (31)	118/124 (93)
p-value	0.88	0.99	0.20	<0.001	0.48

Values in parentheses indicate percentage values.

[1]Of 172 colon cancers tested, 124 were non-MSI-H, and 48 (28%) were MSI-H. DNA did not amplify in 4 of the MSI-H tumors for subsequent target gene testing. Statistical significance was determined utilizing the log rank test.

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TABLE II

TARGET GENE MUTATIONS AND SURVIVAL IN THE DUKES B2 COHORT WITH MSI-H[1]

Target gene	Mutation status	Alive (*N*)	Dead (*N*)	*p*-value
TGFBR2 (*N* = 45)	Wild type	7	3	0.17
	Mutated	31	4	
BAX (*N* = 47)	Wild type	14	4	0.15
	Mutated	27	2	
Both *TGFBR2* and *BAX* (*N* = 44)	None or either gene mutated	15	5	0.05
	TGFBR2 mutated, *BAX* wild type	8	2	
	BAX mutated, *TGFBR2* wild type	3	1	
	TGFBR2 wild Type, *BAX* wild type	4	2	
	TGFBR2 mutated, *BAX* mutated	23	1	

[1] Statistical significance was determined utilizing univariate analysis with the log rank test. *TGFBR2* and *BAX* were compared as mutation positive *versus* mutation absent. The combined *TGFBR2* and *BAX* group was compared as both genes mutated *versus* all other combinations. Other individual comparisons within the combined *TGFBR2* and *BAX* group did not show statistical significance. *p* value is determined for mutated *versus* nonmutated scenarios.

TABLE III

CHARACTERISTICS OF NORTH CAROLINA COHORT[1]

	Gender	Average age (years)	Location [proximal colon]	Grade [well to moderate]
MSI-H [*N* = 54]	M23 F31	63.7	32/54 (59)	20/47 (42)
MSS [*N* = 449]	M251 F198	63.0	157/449 (35)	355/426 (83)
p-value	0.06	0.90	<0.001	<0.001

Values in parentheses indicate percentage values.

[1]Of 503 colon cancers tested, 449 were non-MSI-H, and 54 (11%) were MSI-H. Survival data was not available for this cohort. Statistical significance was determined utilizing the χ^2 test.

TABLE IV

TARGET GENE MUTATION PROFILE IN MSI-H COLON CANCERS WITH KNOWN STAGE FROM THE NORTH CAROLINA COHORT WITH MSI-H (*N* = 41)[1]

Mutated target gene	*ACVR2* mutated [*N* = 32]	*ACVR2* WT (*N* = 9)	*TGFBR2* mutated (*N* = 28)	*TGFBR2* WT (*N* = 13)	*BAX* mutated (*N* = 17)	*BAX* WT (*N* = 24)	Combined *ACVR2/BAX/ TGFBR2* mutated (*N* = 12)	Combined *ACVR2/BAX/ TGFBR2* WT (*N* = 5)
Dukes A + B	14/32 (44)	2/9 (22)	12/28 (43)	4/13 (31)	7/17 (41)	10/24 (42)	5/12 (42)	2/5 (40)
Dukes C + D	18/32 (56)	7/9 (78)	16/28 (57)	9/13 (69)	10/17 (59)	14/24 (58)	7/12 (58)	3/5 (60)
p-value	0.43		0.46		0.98		0.95	

Values in parentheses indicate percentage values.

[1] A, B, C and D refer to the Astler–Collier–Dukes stage. Statistical significance was determined utilizing the χ^2 test.

TABLE V

ACVR2, BAX, AND *TGFBR2* MUTATIONAL STATUS IN MSI-H COLON CANCERS WITH KNOWN GRADE [*ACVR2*, *n* = 47; *BAX*, *n* = 49; *TGFBR2*, *n* = 49] OR SIZE [*ACVR2*, *n* = 28; *BAX*, *n* = 25; *TGFBR2*, *n* = 23] FROM THE NCCCS COHORT

Variable	*ACVR2* wild type	*ACVR2* mutation	*p*-value	*BAX* wild type	*BAX* mutation	*p*-value	*TGFBR2* wild type	*TGFBR2* mutation	*p*-value
Grade *(N)*	*n* = 7	*n* = 40		*n* = 27	*n* = 22		*n* = 17	*n* = 32	
W + M	6	14		18	12		13	19	
Poor	1	26	<0.01	9	10	NS	4	13	NS
Volume *(N)*	*n* = 8	*n* = 20		*n* = 17	*n* = 7		*n* = 8	*n* = 15	
Mean (cm^3)	35	99		60	70		76	48	
Median (cm^3)	13	47	<0.05	14	14	NS	25	25	NS

W+M refers to well and moderately differentiated tumors. Statistical significance was determined by χ^2 (grade) and Student *t*-test (tumor volume).