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KRAS Mutation and Microsatellite Instability: Two Genetic Markers of Early Tumor Development That Influence the Prognosis of Colorectal Cancer

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Abstract

Introduction—We examined two genetic markers established early in colorectal tumor development, microsatellite instability (MSI) and mutation of the KRAS proto-oncogene, to see if these genetic changes influence metastatic disease progression and survival.

Patients and methods—MSI and KRAS mutation status were assessed in 532 primary adenocarcinomas (stage I–IV) from patients treated by colon resection. Median follow-up was 4.1 years (range 0–13.3 years) overall, 5.4 years for survivors.

Results—MSI and *KRAS* mutation were detected in 12 and 36% of cases, respectively. MSI was more common in early-stage disease (I, 15%; II, 21%; III, 10%; IV, 2%; $P = 0.0001$). Prevalence of *KRAS* mutation did not vary with stage (I, 36%; II, 34%; III, 35%; IV, 40%; P = ns). Disease-specific survival was far superior for MSI tumors than for microsatellite stability (MSS) tumors (5-year survival 92 vs. 59%, $P < 0.0001$). KRAS mutation was a marker of poor survival (5-year survival 55 vs. 68%, $P = 0.0002$). Using Cox regression analysis MSI, KRAS mutation, and stage were strong independent predictors of survival in the entire patient population. A high-mortality group with MSS/*KRAS*-mutant tumors was identified within the stage I and II cohort.

Conclusions—MSI and KRAS mutation provide fundamental genetic signatures influencing tumor behavior across patient subsets and stages of tumor development.

The variable cure rate of colorectal cancer offers an excellent clinical model for studying factors influencing metastatic progression. Following surgical resection of the colon, local tumor relapse is rare.[1] Cancer recurrence and death from disease are nearly always due to distant metastases, and are thus determined by tumor biology rather than by variations in presentation or local therapy.

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Extent of disease at the time of diagnosis has a major impact on surgical cure rate, which varies from 90 to 50% for cancers that are clinically localized (stages I–III, respectively) to less than 10% for cancers that have metastasized to distant organs (stage IV).[2–5] These data support a developmental model in which distant metastases are generally established only after a substantial period of local growth and invasion. Thus the determinants of surgical cure are, first, the time elapsed from cancer initiation to surgical treatment and, second, the speed with which a cancer establishes viable micrometastases in distant organs. The discovery and validation of genetic markers determining the efficiency of metastatic progression of colon cancer is therefore an important area of research, with potential value in disease management and basic investigation.

Previous studies have evaluated a variety of genetic changes that appear to influence prognosis, including microsatellite instability, p53 mutation, KRAS mutation, aneuploidy, 17p loss, 18q loss, 8p loss, and, more recently, patterns of global gene expression and sensitivity to chemotherapeutic agents.[6–13] Because of the complexity of both tumor biology and clinical management, study of a large number of cases is essential to successful evaluation of even a single prognostic marker. Other important elements include quality control of the genetic analyses, accuracy of clinical staging, quality of surgical treatment, and adequacy of patient follow-up. Furthermore, when evaluating the impact of tumor biology on prognosis, use of disease recurrence or disease-specific survival as a primary endpoint is preferable to use of overall survival. Because of these numerous pitfalls, published studies have presented uncertain, and at times conflicting, messages about the value of genetic markers in determining prognosis in colorectal cancer.[14–16] Accordingly, genetic markers have yet to penetrate clinical management of primary colorectal cancer despite widespread acknowledgment of their potential value.[17–19]

We have examined two prevalent and well-studied genetic markers that are acquired very early in the development of colorectal neoplasia: microsatellite instability (MSI) and KRAS mutation. MSI defines a class of colon cancers with a high rate of mutations in repeat sequences due to a defect in DNA mismatch repair.[20,21] The onset of MSI happens very early in colon cancer development; once established, MSI has a dominant effect on cancer phenotype.[22–24] Three large studies have demonstrated the favorable prognosis associated with MSI in colorectal cancers.[8,9,25] Though there is a five-microsatellite marker assay, initially recommended by the National Cancer Institute in 1997 (NCI assay), data from our and other laboratories have shown that more specific identification of MSI can be achieved with the use of assays focusing on mononucleotide markers.[26–29] In this series we used our previously validated three-marker assay.[26] This three-marker assay utilizes two mononucleotide markers (BAT25 and BAT26) and one dinucleotide tie-breaker (D2S123).

Mutated *KRAS* is a powerful transforming oncogene that activates a multitude of specific effector molecules such as Raf, PI3 Kinase, Phospholipase C, and Ral, disrupting many cell functions including cell proliferation, cytoskeletal organization, motility, and apoptosis.[30,31] *KRAS* remains among the most common mutations found in human cancers.[32] These mutations have been detected in the earliest neoplastic lesions found in colonic mucosa, and appear to exert a strong influence on the growth of polyps and early cancers.[33,34] Furthermore, *KRAS* mutations have been correlated with methylation phenotype and

inversely with MSI, and thus may indirectly identify tumors with distinct forms of genetic instability.[35] The importance of mutated *KRAS* as a prognostic marker is controversial. Several large studies have demonstrated that particular *KRAS* mutations impact survival, though none have demonstrated prognostic value independent of stage.[14,36,37] The association between *KRAS* mutation and the absence of response to cetuximab is now well documented. However, the patients in this study were accrued prior to the clinical use of cetuximab; thus there will be no interaction between *KRAS* mutation, cetuximab exposure, and survival.

We reasoned that, if the genetic basis for colon tumor progression is established early and sustained through tumor development, these two markers would likely demonstrate an independent and measurable correlation with the late stages of cancer progression. Therefore, we studied MSI and *KRAS* status in a large series of colon cancer patients treated in the 1990s at one specialty center where staging, surgical resection, and adjuvant therapy were highly consistent. Our aim was to define the relationship of these genetic markers to metastatic disease progression and survival.

PATIENTS AND METHODS

Patients and Samples

Tumor and normal tissue samples were collected under Institutional Review Board protocol from patients undergoing elective surgery for colorectal cancer at Memorial Sloan–Kettering Cancer Center. The operations were performed between January 1990 and December 1997. Colon cancer had greater representation in this series compared with rectal cancer: 400 and 132, respectively. Rectal cancers that received preoperative radiotherapy were excluded. There were 54 patients with distal rectal cancer (within 6 cm of the anal verge). All patients were staged preoperatively with computed tomography (CT) scans and chest X-rays, and all underwent colon resection as their initial cancer treatment. Final tumor–node–metastasis (TNM) stage was assigned using the American Joint Committee on Cancer staging manual and was based on CT findings, intraoperative findings, and final pathology reports. All patients underwent radical resection of the primary tumor. The stage IV group was a combination of patients undergoing palliative colon resection (73%) or potentially curative metastasis resection (27%). The majority of patients with stage III (77%) and stage IV (88%) cancers received 5-flourouracil (5-FU)-based chemotherapy post-operatively. Chemotherapy was not used for stage I cancer and rarely for stage II (11%).

Colon tumor tissue and normal mucosa were obtained at time of surgical resection from 532 patients and snap-frozen in liquid nitrogen. DNA was first extracted and purified using a proteinase potassium/lithium chloride/ethanol (K/ LiCl/EtOH) protocol, then quantified using $OD_{260/280}$ with a GeneQuant *pro*™ DNA calculator. Median patient age was 67 years (range 23–93 years). Two hundred seventy-three patients were male and 259 were female. Median follow-up after colon resection was 4.1 years (range 0–13.3 years). Survivors were followed for a median of 5.4 years (range 0–13.3 years).

Microsatellite Instability (MSI)

MSI analysis was performed on matched tumor and normal tissue (100 ng DNA per reaction) using a previously published multiplex protocol.[26] Oligonucleotide primers for BAT25, BAT26, and D2S123 were fluorescently labeled and amplified simultaneously using AmpliTaqGold® DNA polymerase (Applied Biosystems, Foster City, CA).[38] The polymerase chain reaction (PCR) products were resolved in an ABI PRISM™ 377 DNA Sequencer. MSI was defined as two or three PCR products demonstrating instability consistent with the MSI-H genotype.

Polymerase Chain Reaction/Ligase Detection Reaction (PCR/LDR)

KRAS mutation status in tumors was assessed using a previously published polymerase chain reaction and ligase detection reaction (PCR/LDR) that can detect 1 mutant *KRAS* allele among 200 wild-type alleles and that, in our hands, is slightly more accurate than DNA sequencing.[39] Oligonucleotide primers and Taq DNA polymerase were used to amplify *KRAS* exon 1. Wild-type primers for *KRAS* exon 30 and mutation-specific primers for codons 12 and 13 were used in the LDR.[39] The LDR products were resolved on 12.5% polyacrylamide gel in an ABI PRISM™ 377 DNA sequencer. Specific mutations in codons 12 and 13 were identified by their corresponding ligation products.

Statistics

Pearson's chi-squared and Fisher's exact tests were applied to the results, as appropriate. Analysis of variance (ANOVA) was used to analyze MSI prevalence by stage. Survival curves were generated by the Kaplan–Maier method and subjected to the log-rank test. Multivariable analysis was performed using Cox regression. All reported *P* values are two-sided, and *P* values of < 0.05 were considered significant.

RESULTS

Frequency

MSI status was evaluated in 478 cases with matched tumor and normal tissue. The other 54 cases had insufficient normal tissue for MSI analysis. MSI was detected in 58 cases (12%). *KRAS* mutation status was documented in 531 cases. The *KRAS* gene failed to PCR amplify in one tumor due to insufficient tumor DNA. One or more *KRAS* mutations were detected in 190 tumors (36%). Of these, 157 tumors had codon 12 mutations (67 were aspartate-12, 47 were valine-12, 15 were alanine-12, 15 were cysteine-12, 7 were serine-12, 3 were arginine-12), and 33 had codon 13 mutations (all were aspartate-13).

Patient Characteristics

Neither MSI nor *KRAS* mutations were associated with patient age or gender (data not shown).

Tumor Characteristics

MSI was more common in early-stage cancers ($P < 0.0001$), whereas prevalence of *KRAS* mutation did not vary with stage (Fig. 1a). Consistent with prior studies, MSI was strongly

associated with tumor location proximal to the splenic flexure (38 of 58 cases, $P < 0.0001$), poorly differentiated cancers (18 of 58 cases, $P < 0.001$), and mucinous cancers (38 of 58, $P < 0.0001$), and was inversely correlated with presence of *KRAS* mutation (Fig. 1b). Cancers with *KRAS* mutation had a higher likelihood of mucinous histology (78 of 190, $P = 0.003$). Neither *KRAS* mutation nor MSI were associated with location in the rectum (P [0.40).

Survival

MSI was a favorable marker of survival compared with those tumors that had MSS (5-year survival 92 vs. 59%, $P < 0.0001$) (Fig. 2a). *KRAS* was an unfavorable marker of survival (5-year survival 55 vs. 68%, $P = 0.0002$) (Fig. 2b). Among patients with MSI tumors there was a trend towards better survival in stages I and IV, and a significant survival difference in stage II (Fig. 3a). Among patients with *KRAS* mutant tumors, there was a strong trend towards worse survival in stages I and IV, and a significant survival difference in stages II and III (Fig. 3b). Survival analyses were performed on the individual codon 12 and 13 mutations. There were no significant survival differences seen between the individual mutations.

Combining MSI and *KRAS*

Survival was analyzed for the four groups identified by these two genetic markers (Fig. 4). Each marker was found to exert a consistent impact on prognosis irrespective of the status of the other marker. The group with both markers favorable (MSI and wild-type *KRAS*, $n = 45$) had the best survival (95% at 5 years), whereas the group with both markers unfavorable (MSS and mutant *KRAS*, $n = 157$) had the worst survival (51% at 5 years). Among the earlystage cancers (stages I and II, $n = 222$) expected to have an excellent prognosis, those patients with MSS and mutant *KRAS* identified in their tumors ($n = 66$) had significantly worse survival compared with all other stage I and II patients (85 vs. 96% at 5 years, 64 vs. 92% at 7 years, $P = 0.0005$) (Fig. 5).

Multivariable Regression Analysis

To determine their prognostic value independent of disease stage, MSI and *KRAS* mutation were entered into a Cox regression model (Table 1). TNM stage, *KRAS* mutation, and MSI were found to be independently associated with disease-specific survival. MSI was associated with a fivefold reduction in risk of cancer death, whereas *KRAS* was associated with a 1.75-fold increase in risk of cancer death.

DISCUSSION

Our data demonstrate that genetic events established early in tumor development have a powerful effect on metastatic progression of colorectal cancer. In our large series of surgical patients with primary adenocarcinoma of the colon or rectum, 37% died of cancer. MSI was linked to only 2% of cancer deaths, and multivariable models including stage of disease predicted a fourfold reduction in actuarial risk of cancer mortality. Conversely, *KRAS* mutation was linked to 47% of all cancer deaths and predicted a nearly twofold increase in cancer mortality risk. In every stage of disease and in nearly every patient stratum, one or both of these markers identified patients with better (MSI) or worse (*KRAS* mutation)

prognosis. These data indicate that MSI and *KRAS* mutation are biomarkers which distinguish genetic subsets of colorectal cancer that differ in speed and efficiency of metastatic spread. MSI tumors rarely progress to metastasis, whereas MSS tumors with *KRAS* mutation progress to metastasis in greater than 50% of cases.

Evidence from other investigators supports the idea that MSI and *KRAS* mutation are genetic markers that are established early and remain biologically relevant throughout all stages of tumor development. Both MSI and *KRAS* mutation have been found in aberrant crypt foci, the earliest neoplastic lesions that can be identified in the colon.[40,41] Topographic sampling of colon cancers arising within colon adenomas has shown that, when present in an adenoma, both MSI and *KRAS* mutation are stable and clonally expanded within the cancer.[34,42] In addition, *KRAS* mutations found in primary colon cancers are preserved in recurrences and metastases.[43]

Our data convincingly show that MSI and *KRAS* status each provide unique and complementary information about prognosis (Fig. 4). We believe that, when used in combination, these markers constitute a starting point for developing a molecular prognostic scoring system for early-stage colorectal cancer. The multivariable model demonstrates that both markers are predictors of outcome independent of stage. However, when stratifying by individual stage, some statistical power is lost due to smaller sample size (Figs. 3, 4). Nevertheless, in this series, stage I and II patients (n = 222) had an overall 7-year disease-specific survival of 84%. Because of this overall good prognosis, high-risk patients were hard to identify on clinical grounds and adjuvant chemotherapy was rarely used. In this population, MSI and *KRAS* mutation are common (stage I: 15 and 36%, respectively; stage II: 22 and 34%, respectively). In combination, the markers were capable of identifying a high-risk subset within the stage I and II cohort (MSS/*KRAS* mutation, n = 66) representing only 30% of the early-stage patients but 14 of 25 (56%) of the cancer deaths. These two markers provided excellent stratification of prognosis in stage I and II patients: 64% survival at 7 years for the high-risk group versus 92% for the low-risk group. Useful prognostic information about both groups can be provided by these markers (Fig. 5). The clinical utility of these markers was further supported by multivariate analysis of the entire cohort showing that MSI and *KRAS* have prognostic power similar to that of lymph node status, which is currently the standard used to select patients for adjuvant chemotherapy (Table 1).

The link between favorable prognosis and MSI status is well supported in the literature.[7,8,25] However, previously published data on *KRAS* mutation is inadequate for drawing conclusions about prognosis. Most studies evaluating *KRAS* mutation as a prognostic marker have been severely underpowered.[44,45] A meta-analysis of data from 22 centers published in 1998 and a follow-up meta-analysis of data from 35 centers published in 2001 concluded that *KRAS* mutation predicts poor survival in colorectal cancer patients, although the most recent study limited this conclusion to the valine-12 mutation.[14,15] Unfortunately, both meta-analyses were limited by data that was heterogenous with regard to patient accrual, experimental method, marker prevalence, and clinical follow-up.

Only three groups have previously reported data on the prognostic implications of both markers simultaneously in colorectal cancer.[46–48] None demonstrated meaningful

relationships between the markers and survival. Our series is the largest study of MSI and *KRAS* mutation in the same cohort, and we were able to perform an adequately powered analysis. Additionally we used well-validated assays for MSI and *KRAS* mutation, and marker prevalence was highly consistent with that demonstrated by other well-controlled studies.[26,39]

There are data that show MSI or *KRAS* mutation is associated with in vitro and in vivo variation in response to chemotherapy.[49–53] However, this retrospective study is not designed to address the interaction between these molecular changes, chemotherapy, and outcome. The finding by univariable analysis that patients who were exposed to chemotherapy had significantly worse survival should be considered in conjunction with the fact that patients with stage III and IV colorectal cancer (CRC) were routinely treated with postoperative chemotherapy and those with stage I and II CRC were routine treated with surgery alone. This difference disappeared in the multivariable model. However, confounding by indication would obviously occur and therefore we cannot comment on possible effect modification, the greater or lesser impact of *KRAS* mutation or MSI in the chemotherapy and non-chemotherapy groups. Nevertheless, given that none of these patients received cetuximab during the study period one may exclude the possibility that the differences in survival are due to *KRAS*-mutant tumor resistance to cetuximab.

Our study provides strong evidence that likelihood of metastatic progression in colorectal cancer can be estimated based on biomarkers present in the primary tumor. Knowledge of MSI and *KRAS* status may enhance clinicopathologic staging in colorectal cancer patients who are staged and treated in a consistent manner. Validation of additional prognostic markers promises to provide a panel of genetic markers that will help refine management decisions for individual patients based on tumor biology.

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REFERENCES

1. Skibber, J.; Minsky, B.; Hoff, P. Cancer of the colon. In: DeVita, VT.; Rosenberg, SA., editors. Principles and practice of oncology. Lippincott Williams & Wilkins; Philadelphia: 2001.
2. Chapuis PH, Fisher R, Dent OF, Newland RC, Pheils MT. The relationship between different staging methods and survival in colorectal carcinoma. Dis Colon Rectum. 1985; 28:158–61. [PubMed: 3971820]
3. Barillari P, de Angelis R, Valabrega S, Indinnimeo M, Gozzo P, Ramacciato G, et al. Relationship of symptom duration and survival in patients with colorectal carcinoma. Eur J Surg Oncol. 1989; 15:441–5. [PubMed: 2551743]
4. Robinson M, Hardcastle J. Should we be screening for colorectal cancer? Br Med Bull. 1998; 54:807–821. [PubMed: 10367416]
5. Culliford, A.; Paty, P. Surgery of colon cancer. In: Saltz, L., editor. Colorectal cancer: multimodality management. Blackwell; Oxford, UK: 2002.
6. Diep CB, Thorstensen L, Meling GI, Skovlund E, Rognum TO, Lothe RA. Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. J Clin Oncol. 2003; 21:820–9. [PubMed: 12610180]

7. Elsaleh H, Powell B, McCaul K, Grieu F, Grant R, Joseph D, et al. P53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin Cancer Res. 2001; 7:1343–9. [PubMed: 11350904]
8. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000; 342:69–77. [PubMed: 10631274]
9. Halling KC, French AJ, McDonnell SK, Burgart LJ, Schaid DJ, Peterson BJ, et al. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. J Natl Cancer Inst. 1999; 91:1295–303. [PubMed: 10433618]
10. Peltomäki P, Lothe RA, Aaltonen LA, Pylkkänen L, Nyström-Lahti M, Seruca R, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res. 1993; 53:5853–5. [PubMed: 8261393]
11. Slattery ML, Curtin K, Schaffer D, Anderson K, Samowitz W. Associations between family history of colorectal cancer and genetic alterations in tumors. Int J Cancer. 2002; 97:823–7. [PubMed: 11857362]
12. Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001; 344:1196–206. [PubMed: 11309634]
13. Liévre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, et al. *KRAS* mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol. 2008; 26:374–9. [PubMed: 18202412]
14. Andreyev HJ, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer. 2001; 85:692–6. [PubMed: 11531254]
15. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter ''RASCAL'' study. J Natl Cancer Inst. 1998; 90:675–84. [PubMed: 9586664]
16. Klump B, Hehls O, Okech T, Hsieh CJ, Gaco V, Gittinger FS, et al. Molecular lesions in colorectal cancer: impact on prognosis? Original data and review of the literature. Int J Colorectal Dis. 2004; 19:23–42. [PubMed: 12827409]
17. Duffy MJ, van Dalen A, Haglund C, Hansson L, Holinski-Feder E, Klapdor R, et al. Tumour markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines for clinical use. Eur J Cancer. 2007; 43:1348–60. [PubMed: 17512720]
18. Duffy MJ, van Dalen A, Haglund C, Hansson L, Klapdor R, Lamerz R, et al. Clinical utility of biochemical markers in colorectal cancer: European Group on Tumour Markers (EGTM) guidelines. Eur J Cancer. 2003; 39:718–27. [PubMed: 12651195]
19. Graziano F, Cascinu S. Prognostic molecular markers for planning adjuvant chemotherapy trials in Dukes' B colorectal cancer patients: how much evidence is enough? Ann Oncol. 2003; 14:1026–38. [PubMed: 12853343]
20. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature. 1993; 363:558–61. [PubMed: 8505985]
21. Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. Curr Opin Genet Dev. 1999; 9:89–96. [PubMed: 10072354]
22. Pedroni M, Sala E, Scarselli A, Borghi F, Menigatti M, Benatti P, et al. Microsatellite instability and mismatch-repair protein expression in hereditary and sporadic colorectal carcinogenesis. Cancer Res. 2001; 61:896–9. [PubMed: 11221877]
23. Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. Nat Rev Cancer. 2003; 3:695–701. [PubMed: 12951588]
24. Yamashita K, Dai T, Dai Y, Yamamoto F, Perucho M. Genetics supersedes epigenetics in colon cancer phenotype. Cancer Cell. 2003; 4:121–31. [PubMed: 12957287]
25. Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001; 10:917–23. [PubMed: 11535541]

26. Nash GM, Gimbel M, Shia J, Culliford AT, Nathanson DR, Ndubuisi M, et al. Automated, multiplex assay for high-frequency microsatellite instability in colorectal cancer. J Clin Oncol. 2003; 21:3105–12. [PubMed: 12915601]
27. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology. 2002; 123:1804–11. [PubMed: 12454837]
28. Xicola RM, Llor X, Pons E, Castells A, Alenda C, Piñol V, et al. Performance of different microsatellite marker panels for detection of mismatch repair-deficient colorectal tumors. J Natl Cancer Inst. 2007; 99:244–52. [PubMed: 17284719]
29. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004; 96:261–8. [PubMed: 14970275]
30. Cox AD, Der CJ. Ras family signaling: therapeutic targeting. Cancer Biol Ther. 2002; 1:599–606. [PubMed: 12642680]
31. Sahai E, Olson MF, Marshall CJ. Cross-talk between Ras and Rho signalling pathways in transformation favours proliferation and increased motility. EMBO J. 2001; 20:755–66. [PubMed: 11179220]
32. Porta M, Ayude D, Alguacil J, Jariod M. Exploring environmental causes of altered ras effects: fragmentation plus integration? Mol Carcinog. 2003; 36:45–52. [PubMed: 12557259]
33. Cheng L, Lai MD. Aberrant crypt foci as microscopic precursors of colorectal cancer. World J Gastroenterol. 2003; 9:2642–9. [PubMed: 14669304]
34. Shibata D, Schaeffer J, Li ZH, Capella G, Perucho M. Genetic heterogeneity of the c-K-ras locus in colorectal adenomas but not in adenocarcinomas. J Natl Cancer Inst. 1993; 85:1058–63. [PubMed: 8515492]
35. Kondo Y, Issa JP. Epigenetic changes in colorectal cancer. Cancer Metastasis Rev. 2004; 23:29–39. [PubMed: 15000147]
36. Ahnen DJ, Feigl P, Quan G, Fenoglio-Preiser C, Lovato LC, Bunn PA Jr. et al. Ki-ras mutation and p53 overexpression predict the clinical behavior of colorectal cancer: a Southwest Oncology Group study. Cancer Res. 1998; 58:1149–58. [PubMed: 9515799]
37. Samowitz WS, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. Cancer Epidemiol Biomarkers Prev. 2000; 9:1193–7. [PubMed: 11097226]
38. Umetani N, Sasaki S, Watanabe T, Ishigami H, Ueda E, Nagawa H. Diagnostic primer sets for microsatellite instability optimized for a minimal amount of damaged DNA from colorectal tissue samples. Ann Surg Oncol. 2000; 7:276–80. [PubMed: 10819367]
39. Khanna M, Park P, Zirvi M, Cao W, Picon A, Day J, et al. Multiplex PCR/LDR for detection of K-ras mutations in primary colon tumors. Oncogene. 1999; 18:27–38. [PubMed: 9926917]
40. Alrawi SJ, Schiff M, Carroll RE, Dayton M, Gibbs JF, Kulavlat M, et al. Aberrant crypt foci. Anticancer Res. 2006; 26:107–19. [PubMed: 16475686]
41. Pretlow TP, Brasitus TA, Fulton NC, Cheyer C, Kaplan EL. K-ras mutations in putative preneoplastic lesions in human colon. J Natl Cancer Inst. 1993; 85:2004–7. [PubMed: 8246286]
42. Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M. Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. Nat Genet. 1994; 6:273–81. [PubMed: 8012390]
43. Losi L, Benhattar J, Costa J. Stability of K-ras mutations throughout the natural history of human colorectal cancer. Eur J Cancer. 1992; 28A:1115–20. [PubMed: 1627381]
44. Finkelstein SD, Sayegh R, Christensen S, Swalsky PA. Genotypic classification of colorectal adenocarcinoma. Biologic behavior correlates with K-ras-2 mutation type. Cancer. 1993; 71:3827–38. [PubMed: 8508351]
45. Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, et al. Allelotype of colorectal carcinomas. Science. 1989; 244:207–11. [PubMed: 2565047]
46. Curran B, Lenehan K, Mulcahy H, Tighe O, Bennett MA, Kay EW, et al. Replication error phenotype, clinicopathological variables, and patient outcome in Dukes' B stage II (T3,N0,M0) colorectal cancer. Gut. 2000; 46:200–4. [PubMed: 10644313]

47. Gnanasampanthan G, Elsaleh H, McCaul K, Iacopetta B. Ki-ras mutation type and the survival benefit from adjuvant chemotherapy in Dukes' C colorectal cancer. J Pathol. 2001; 195:543–8. [PubMed: 11745689]

48. Wang C, van Rijnsoever M, Grieu F, Bydder S, Elsaleh H, Joseph D, et al. Prognostic significance of microsatellite instability and Ki-ras mutation type in stage II colorectal cancer. Oncology. 2003; 64:259–65. [PubMed: 12697967]

49. Rodriguez R, Hansen LT, Phear G, Scorah J, Spang-Thomsen M, Cox A, et al. Thymidine selectively enhances growth suppressive effects of camptothecin/irinotecan in MSI? cells and tumors containing a mutation of MRE11. Clin Cancer Res. 2008; 14:5476–83. [PubMed: 18765539]

50. Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. Clin Cancer Res. 1998; 4:1–6. [PubMed: 9516945]

51. Liévre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. *KRAS* mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006; 66:3992–5. [PubMed: 16618717]

52. Fallik D, Borrini F, Boige V, Viguier J, Jacob S, Miquel C, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res. 2003; 63:5738–44. [PubMed: 14522894]

53. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of *KRAS* and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR 21. J Clin Oncol. 2008; 26:4268–75. [PubMed: 18626007]

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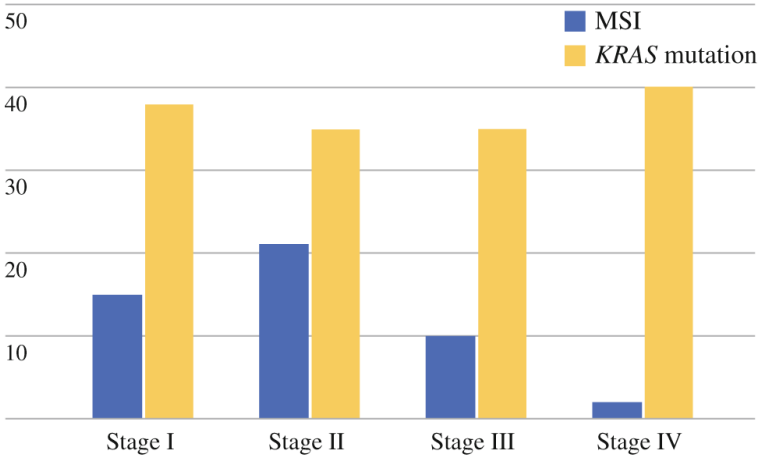

b

	MSI n (%)	MSS n (%)
KRAS wild type	45 (78)	261 (62)
KRAS mutation	12 (22)	157 (38)

Chi-square p-value = 0.02

FIG. 1.

a Stage distribution of MSI ($P < 0.0001$) and *KRAS*. b Chisquare table of MSI and *KRAS* prevalence

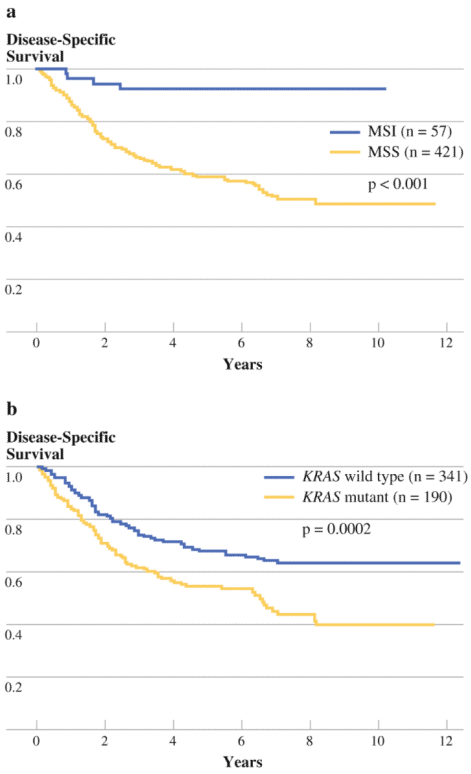

FIG. 2.

Disease-specific survival for stage I–IV patients: a MSI versus MSS, b *KRAS* mutant versus wild type

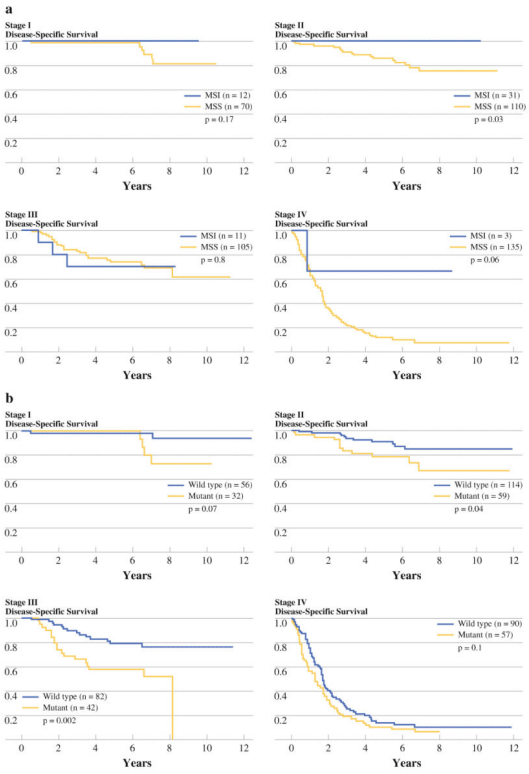

FIG. 3.

Disease-specific survival by stage: a MSI versus MSS, b *KRAS* mutant (mut) versus Wild type (wt)

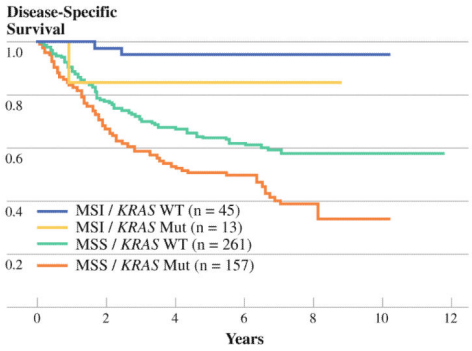

FIG. 4 Disease-specific survival stratified by both genetic markers

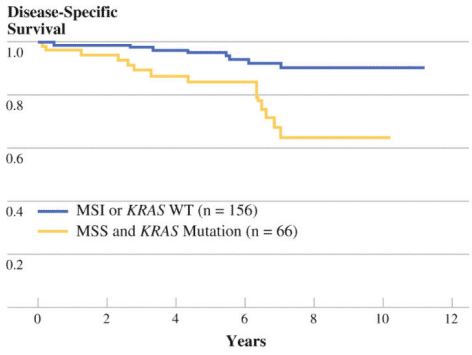

FIG. 4.

Disease-specific survival stratified by both genetic markers

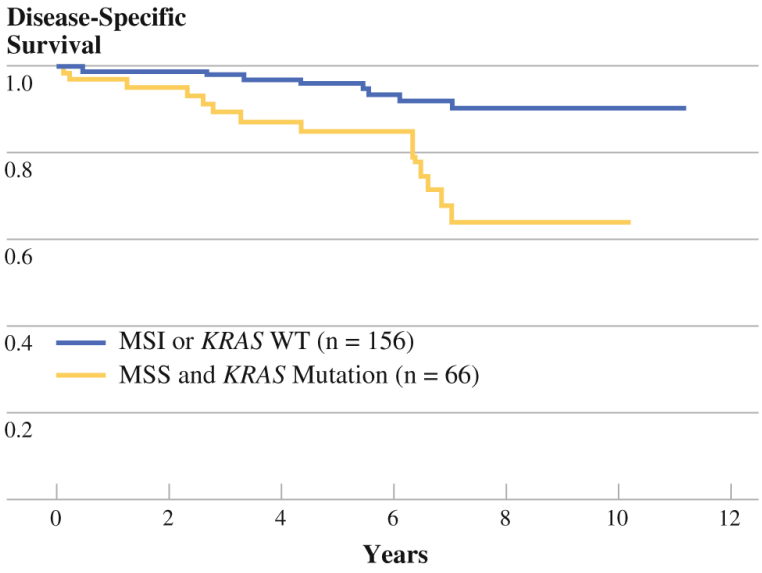

FIG. 5.
Early-stage cancer stratified by MSS and mutant *KRAS*

TABLE 1

Univariable (log rank) and multivariable (Cox regression) analysis of clinical, pathological, and molecular variables

Variable	Univariable analysis[a]			Multivariable analysis[b]		
	n	7-year DSS (%)	*P*-Value	Hazard ratio	95% CI	*P*-Value
Men	*111*	54	0.07			
Women	262	61				
Age ≤ 67 years	251	55	0.17			
Age ≥ 67 years	283	61				
Proximal to splenic flexure	198	60	0.87			
Distal to splenic flexure	336	57				
Tumor grade I	25	60	0.75			
Tumor grade II	432	58				
Tumor grade III	77	58				
Mucinous cell type	76	59	0.78			
Nonmucinous cell type	458	57				
PNI present	41	48	0.0004	1.38	0.71–2.70	0.34
PNI absent	349	63				
LVI present	109	46	0.0004	1.07	0.64–1.77	0.80
LVI absent	406	60				
Stage IV versus I	147	8	<0.0001	25.6	9.1–71.4	<0.0001
Stage III versus I	124	67		9.4	5.2–17.2	
Stage II versus I	171	80		6.2	3.7–10.5	
Stage I	92	92				
Chemotherapy	178	44	<0.0001	1.04	0.55–1.98	0.91
No chemotherapy	212	78				
Preop CEA > 5.0 ng/ml	179	42	0.001	1.22	0.73–2.04	0.46
Preop CEA normal	252	80				
MSI	58	92	<0.0001	0.18	0.06–0.60	0.005
MSS	420	53				
KRAS mutant	190	46	0.0002	1.75	1.15–2.67	0.009
KRAS wild type	342	64				

LVI lymphovascular invasion, *PNI* perineural invasion, *CEA* carcinoembryonic antigen, *Preop* preoperative

[a] Kaplan–Meier with *P*-values calculated by log-rank test

[b] Cox regression (backwards stepwise)