The American Journal of Surgery (2013) 205, 608-612

The American Journal of Surgery®

North Pacific Surgical Association

Stage III & IV colon and rectal cancers share a similar genetic profile: a review of the Oregon Colorectal Cancer Registry

Ute Gawlick, M.D., Ph.D.[a], Kim C. Lu, M.D.[b,*], Miriam A. Douthit, M.A.[b], Brian S. Diggs, Ph.D.[b], Kathryn G. Schuff, M.D.[c], Daniel O. Herzig, M.D.[b], Vassiliki L. Tsikitis, M.D.[b]

[a]Intermountain Healthcare, Murray, UT, USA; [b]Department of Surgery and Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA; [c]Division of Endocrinology, Department of Internal Medicine, Oregon Health and Science University, Portland, OR, USA

KEYWORDS:
Colorectal cancer;
BRAF mutation;
KRAS mutation;
MET mutation;
NRAS mutation;
PIK3CA mutation

Abstract

BACKGROUND: Determining the molecular profile of colon and rectal cancers offers the possibility of personalized cancer treatment. The purpose of this study was to determine whether known genetic mutations associated with colorectal carcinogenesis differ between colon and rectal cancers and whether they are associated with survival.

METHODS: The Oregon Colorectal Cancer Registry is a prospectively maintained, institutional review board–approved tissue repository with associated demographic and clinical information. The registry was queried for any patient with molecular analysis paired with clinical data. Patient demographics, tumor characteristics, microsatellite instability status, and mutational analysis for p53, *AKT*, *BRAF*, *KRAS*, *MET*, *NRAS*, and *PIK3CA* were analyzed. Categorical variables were compared using chi-square tests. Continuous variables between groups were analyzed using Mann-Whitney *U* tests. Kaplan-Meier analysis was used for survival studies. Comparisons of survival were made using log-rank tests.

RESULTS: The registry included 370 patients: 69% with colon cancer and 31% with rectal cancer. Eighty percent of colon cancers and 68% of rectal cancers were stages III and IV. Mutational analysis found no significant differences in detected mutations between colon and rectal cancers, except that there were significantly more *BRAF* mutations in colon cancers compared with rectal cancers (10% vs 0%, $P < .008$). No differences were seen in 5-year survival rates of patients with colon versus rectal cancers when stratified by the presence of *KRAS*, *PIK3CA*, and *BRAF* mutations.

CONCLUSIONS: Stage III and IV colon and rectal cancers share similar molecular profiles, except that there were significantly more *BRAF* mutations in colon cancers compared with rectal cancers.

Colon and rectal cancers are staged based only on the depth of tumor penetration, lymph node status, and clinical factors, including the presence of metastases. Molecular analysis does not currently alter staging, although it can be

The authors declare no conflicts of interest.

* Corresponding author. Tel.: +1-503-494-4373; fax: +1-503-494-8884.
E-mail address: luk@ohsu.edu

Manuscript received November 18, 2012; revised manuscript January 22, 2013

0002-9610/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.amjsurg.2013.01.029

used in treatment decisions for both colon and rectal cancers. Although it is thought that colon and rectal cancers may have divergent oncologic behavior, little is known about this behavior on the molecular basis. Increasing evidence supports that the prognosis of colorectal adenocarcinoma is related to genetic and epigenetic factors, which may ultimately contribute to survival.[1–4] Furthermore, the molecular profile of the primary tumor can differ from the metastatic tumor, which may confer a different susceptibility to adjuvant therapy.[5]

There are multiple recognized distinct genetic pathways to colorectal cancer. These include the chromosomal instability pathway, which is associated with known activating mutations in oncogenes such as *KRAS* and *BRAF*, or inactivation of tumor suppressor genes such as the *APC* gene, as described by Vogelstein et al.[6] The microsatellite instability (MSI) pathway is associated with the loss of expression of mismatch repair genes.[7] Within this group of genes, the most common ones are *MLH1* and *MSH2*. The less common ones include *PMS1*, *PMS2*, *MSH3*, and *MSH6*. Furthermore, the CpG island methylation phenotype is a significant contributor of tumor-suppressor gene inactivation in cancer.[8] Within this pathway, hypermethylation of deoxyribonucleic acid (DNA) promoters rich in CpG repeats leads to gene suppression (eg, *MLH1*) and subsequently contributes to the development of colorectal cancers.[9,10]

In some studies, genetic and epigenetic pathways influence colorectal cancer outcomes and survival. In one study, MSI status has been shown to be an independent predictor of disease-free survival of stage II and III colorectal cancers, whereas *KRAS* and *BRAF* mutations were not found to influence survival.[1] Sanchez et al[2] found that for stage I to III colorectal cancers, MSI-high cancers were associated with a better disease-free survival. Furthermore, Iida et al[4] found that the *PIK3CA* mutation in association with high methylation is associated with a significantly poorer disease-specific survival than the wild type. It may be true that not one distinct genetic factor determines survival and response to treatment, but a combination of multiple factors contributes to the final outcome. We examined whether outcomes in colorectal cancers can be linked to differences in genetic pathways (ie, the chromosomal instability, CpG island methylation phenotype, and MSI pathways).

Methods

Patient information, microsatellite instability, and mutation analysis

An institutional review board–approved study was performed, using the Oregon Colorectal Cancer Registry (OCCR). Patient demographics and tumor characteristics, including MSI and mutations for p53, *AKT*, *BRAF*, *KRAS*, *MET*, *NRAS*, and *PIK3CA*, were analyzed in up to 386 patients. Not all patients had every mutation tested. Mutations were tested on the basis of clinical suspicion by the pathologist or medical oncologist.

Tumor specimens and DNA preparation

Blocks of formalin-fixed, paraffin-embedded tumor tissue, or unstained sections of formalin-fixed, paraffin-embedded tissue, were obtained from the pathology archives of Oregon Health and Science University. The diagnosis in each case was confirmed by a single pathologist. Tumor-rich areas (>80% by comparison with a hematoxylin and eosin–stained slide) were dissected from 5-μm unstained sections, and genomic DNA was extracted, using a QIAamp DNI Mini kit (Qiagen, Valencia, CA), in accordance with the manufacturer's instructions.

Mutation screening

A total of 500 ng formalin-fixed, paraffin-embedded–derived DNA was required to screen the 36-multiplex panel. This solid tumor panel includes all of the assays that are part of the commercially available OncoCarta v01 panel (Sequenom, San Diego, CA), as well as 136 custom-designed assays that are now also commercially available (OncoCarta v02; Sequenom). Sequenom's mass spectrometry–based mutation detection method has been previously published.[11]

Statistical analysis

Comparisons of categorical variables between groups were compared using chi-square tests and are reported as numbers and percentages. Continuous variables were compared between groups using Mann-Whitney *U* tests and are reported as medians and interquartile ranges. Survival was measured from the date of surgery to the date of death (event) or the date of last contact (censored). Survival analyses were done with Kaplan-Meier curves, and comparisons between curves were made using log-rank tests. Missing values, due to mutations not tested or other causes, resulted in cases being eliminated from an analysis on a variable-by-variable basis. Statistical significance was determined at a P value $<.05$. All analyses were performed using R version 2.14.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographics

Using the OCCR, we identified 386 potential patients to include in our study. One hundred sixteen patients (31%) had rectal cancer, and 254 (69%) had colon cancer. The status of the remaining 16 patients was indeterminate. Colon cancers were equally distributed between men and women (49% vs 51%), but rectal cancers were more common in men than in women (66% vs 34%). Three