North Pacific Surgical Association

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

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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. © 2013 Elsevier Inc. All rights reserved.

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
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. Furthermore, the molecular profile of the primary tumor can differ from the metastatic tumor, which may confer a different susceptibility to adjuvant therapy.

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 \( \text{Kras} \) and \( \text{Braf} \), or inactivation of tumor suppressor genes such as the \( \text{APC} \) gene, as described by Vogelstein et al. The microsatellite instability (MSI) pathway is associated with the loss of expression of mismatch repair genes. Within this group of genes, the most common ones are \( \text{Mlh1} \) and \( \text{Msh2} \). The less common ones include \( \text{Pms1} \), \( \text{Pms2} \), \( \text{Msh3} \), and \( \text{Msh6} \). Furthermore, the CpG island methylation phenotype is a significant contributor of tumor-suppressor gene inactivation in cancer. Within this pathway, hypermethylation of deoxyribonucleic acid (DNA) promoters rich in CpG repeats leads to gene suppression (eg, \( \text{Mlh1} \)) and subsequently contributes to the development of colorectal cancers.

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 \( \text{Kras} \) and \( \text{Braf} \) mutations were not found to influence survival. Sanchez et al found that for stage I to III colorectal cancers, MSI-high cancers were associated with a better disease-free survival. Furthermore, Iida et al found that the \( \text{Pik3ca} \) 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, \( \text{Akt} \), \( \text{Braf} \), \( \text{Kras} \), \( \text{Met} \), \( \text{Nras} \), and \( \text{Pik3ca} \), 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-\( \mu \)m 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.

**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%).
hundred forty-five patients were staged, using the 7th
edition of the American Joint Committee on Cancer’s
tumor-node-metastasis staging system. Most patients had
advanced stage disease: 198 colon cancers (80%) and 67
rectal cancers (68%) were stages III and IV. Of the colon
cancers, 202 (80%) received either adjuvant or neoadjuvant
therapy, as did 103 rectal cancers (89%). At the time of this
study, 182 patients with colon cancer (78%) and 87 patients
with rectal cancer (79%) were alive (Table 1).

Genetics: microsatellite stability (MSS), tumor
suppressor genes, and oncogenes

Of the 28 patients tested for MSI, 82% of colon cancers
and 100% of rectal cancers showed MSS. Although there
were no significant differences between detected mutations
of p53, AKT, KRAS, MET, NRAS, and PIK3CA between
colon and rectal cancers, there was a significant difference
in the BRAF mutation ($P < .008$). Of the 223 patients
tested, 10% of patients with colon cancer were positive
for the BRAF mutation, while the mutation was detected
in none of the 69 rectal cancers.

Survival

No differences were seen in 5-year survival rates of
patients with colon and rectal cancers when stratified by the
presence of the KRAS ($P < .7937$), PIK3CA ($P < .9731$),
and BRAF ($P < .3110$) mutations, independent of stage
(Fig. 1). In addition, we did not find any differences in sur-
vival in colon or rectal cancers when stratified by MSS or
other mutations.

On further examination of combinations of mutations,
we found that KRAS and PIK3K mutations were the most
common combination, observed in 29 colon cancers,
whereas KRAS and TP53 mutations were the second most
common combination, seen in 9 colon cancers. However,
we did not observe any worsened survival rates in those
patients with a combination of two mutations (data not
shown). Only 3 of the cohort of 386 patients examined pre-
sented with triple mutations in KRAS, TP53, and PI3K.
Furthermore, no differences in survival were observed when
stratified by MSS and combinations of mutations (data
not shown).

Comments

The purpose of this study was to determine whether
known genetic mutations associated with colorectal

<table>
<thead>
<tr>
<th>Stage</th>
<th>Colon cancer</th>
<th>Rectal cancer</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6% (14)</td>
<td>18% (18)</td>
<td>9% (32)</td>
</tr>
<tr>
<td>II</td>
<td>14% (35)</td>
<td>13% (13)</td>
<td>14% (48)</td>
</tr>
<tr>
<td>III</td>
<td>38% (94)</td>
<td>42% (41)</td>
<td>39% (135)</td>
</tr>
<tr>
<td>IV</td>
<td>42% (104)</td>
<td>27% (26)</td>
<td>38% (130)</td>
</tr>
</tbody>
</table>

Table 1 Stages and locations of tumors in 345 patients
included in the study group

Staging based on the 7th edition of the American Joint Committee
on Cancer tumor-node-metastasis staging system. Stage III and IV
colon and rectal cancers have similar genetic profiles, except in the
case of BRAF mutations. BRAF mutations were found in some colon
cancers, but not in rectal cancers. This did not affect survival.
carcinogenesis differ between colon and rectal cancers and if differences were associated with survival independent of stage. Using the OCCR database, we demonstrated that there were no significant differences in the prevalence of MSI and mutations for p53, KRAS, PIK3CA, AKT, MET, and NRAS in stage III and IV colon and rectal cancers. However, similar to studies in early-stage colorectal cancers, we did find a difference between colon and rectal cancers in the BRAF mutation. None of the rectal cancers tested in the OCCR database tested positive for the BRAF mutation. Because not every patient was tested for every mutation, smaller numbers within each subgroup may be subject to type II statistical error.

Others have found differences in MSS and MSI, which we did not find in our study. This may be due to the smaller number of patients or the higher stages of the cancers we included in our study. Most of our colon and rectal cancers showed MSS, not MSI. Of the ones tested, nearly all were wild type for MSH2, MSH6, MLH1, and PMS2. We did not find a survival advantage related to MSI or MSS or the expression of any mutations tested. This stands in contrast to another study that demonstrated an increased recurrence of tumors with MSS, compared with tumors with MSI. In addition, although it is well accepted that MSI-high tumors demonstrate better survival, this result was not observed in our study, and we speculate that the advanced stage of our tumors overcame the MSI-high protective effect.

The presence of the KRAS mutation in colorectal cancers is an early mutation event and is widely accepted as a predictive marker for response to epidermal growth factor receptor–based treatment. These findings have led to tailoring of chemotherapy regimens toward molecular phenotype and away from location. Prabhallad et al proposed treatment of BRAF-mutant cancers with both BRAF and epidermal growth factor receptor inhibitors. The molecular characteristics of colorectal cancers are thought to change with disease progression. Late-stage cancers differ from early stage cancers in that the genetic profile of the primary tumor can change if further mutations occur. In one study, liver metastasectomy specimens were used to determine KRAS/BRAF genotype. BRAF, not KRAS, was found to be an independent prognostic biomarker in patients with liver metastasis. In 2 other studies, BRAF mutations were associated with a worse overall survival rate in colorectal cancers. Higher rates of BRAF mutation were associated with distal tumor. We did not observe BRAF mutations in the rectal cancers, which may be simply due to the small number of cases evaluated. In addition, no survival differences were observed among the patients with BRAF-mutated colon tumors.

Among the 265 advanced stage III and IV tumors in our study, only 14 patients received epidermal growth factor receptor antibody therapy. With so few patients in the cetuximab and multiple-mutation groups, we may have had a problem with statistical power in this retrospective study.

Within the group of patients with rectal cancer receiving neoadjuvant chemoradiation, testing was done only on final specimens collected after neoadjuvant therapy. We cannot tell if neoadjuvant therapy changed molecular profiles. Furthermore, within this group, there was no correlation between tumor mutations and treatment response.

In conclusion, although a gene signature has been suggested to be associated with tumor behavior and risk for recurrence in stage I and II rectal cancers, we did not distinguish any mutations observed in this group of more advanced tumors that changed prognosis. With the exception of BRAF mutations, we did not observe any significant differences between colon and rectal cancers, a finding consistent with a recent study. Prospective molecular analyses of this group of patients may elucidate the significance of BRAF in colorectal cancer.

References

Discussion

Peter C. Wu, M.D. (Seattle, WA): I applaud the extraordinary efforts of Dr. Lu’s research group to genotype such a large group of patients with powerful molecular sequencing technology and admire their commitment to be at the forefront of personalized medicine. Given the low frequency of biomarker mutations in colorectal cancer and low hazard ratios, the authors correctly point out the sample size and power limitations of their biomarker study and need for even larger prospective studies to demonstrate survival benefits. However, rather than defining prognosis, the true clinical value of cancer biomarkers may be their ability to predict treatment response to conventional and targeted therapies and ultimately guide personalized treatment plans. In addition to forming closer collaborations with oncologists and pathologists, gastrointestinal cancer surgeons will soon need to be familiar with terminology typically reserved to breast surgeons in managing “triple and quadruple-negative tumors.” My questions for the authors are as follows:

What were the limitations in the mutational screening which was described to be very robust in the methodology paper with nearly 98% of samples successfully genotyped whereas in this study even for the best marker, KRAS, 65% of samples (241/370) were able to undergo mutational testing? Is the methodology reproducible and reliable?

Among the 265 advanced stage III and IV tumors that were tested in the Oregon Colorectal Cancer Registry, did any of these patients receive EGFR antibody therapy (ie, cetuximab or panitumimab)? Given current practice guidelines that advise KRAS mutational testing prior to initiating antibody therapy, as well as increasing evidence to support the role of BRAF mutational testing; were you able to identify any predictive treatment value of these mutations?

Since 80% and 89% of colon and rectal cancer patients in this study, respectively, received either adjuvant or neoadjuvant systemic therapy; were you able to identify any correlation between tumor biomarkers and treatment response which has been described in a few large randomized studies and meta-analyses?

What do you believe will be the future role of a colon cancer surgeon as we enter a new era of personalized medicine?