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Colorectal cancer in the very young: a comparative study of tumor markers, pathology and survival in early onset and adult onset patients

Sajid A. Khan [a,b,*], Melinda Morris [c], Kamran Idrees [b], Mark I. Gimbel [b], Shoshana Rosenberg [b], Zhaoshi Zeng [b], Fangyong Li [f], Geliang Gan [f], Jinru Shia [e], Michael P. LaQuaglia [d], Philip B. Paty [b]

[a] Section of Surgical Oncology, Department of Surgery, Yale University School of Medicine, New Haven, CT, United States
[b] Colorectal Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, NY, New York, United States
[c] School of Surgery and Pathology, University of Western Australia, Nedlands, Western Australia, Australia
[d] Pediatric Service, Department of Surgery, Memorial Sloan-Kettering Cancer Center, NY, New York, United States
[e] Colorectal Service, Department of Pathology, Memorial Sloan-Kettering Cancer Center, NY, New York, United States
[f] Yale Center for Analytic Sciences, Yale University School of Medicine, New Haven, CT, United States

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ABSTRACT

Introduction: Colorectal cancer (CRC) diagnosed before age 30 years is a fatal disease whose biology remains poorly understood. To understand its pathogenesis, we compared molecular and clinical data in surgically treated early-age onset and adult onset patients.
Materials and Methods: Clinical data and tumor tissue were collected retrospectively for 94 patients with early-age onset CRC (age ≤30 years) and compared to 275 adult CRC patients (age ≥50 years). Tumor morphology, microsatellite instability (MSI) and stability (MSS), *KRAS* and *BRAF* mutations, and mismatch repair (MMR) expression (*MSH2, MLH1, MSH6, PMS2*) were assessed.
Results: Early-age CRC was distinguished from adult CRC by advanced stage presentation ($P < 0.001$), frequent high grade cancers ($P < 0.001$), and poor prognosis ($P < 0.001$). MSI was associated with favorable survival and MMR loss in both groups. Compared to adults, MSI in early-onset CRC was more prevalent ($P < 0.01$), not tightly linked to *MLH1*/*PMS2* loss, and never associated with *BRAFV600E* mutations ($P < 0.01$). MSS/*BRAFV600E* genotype had poor prognosis and was more prevalent in early-age CRC (9% vs. 3%).
Discussion: Specific genetic subtypes are found at different frequencies in early-age onset and adult onset CRC. Complete absence of the indolent MSI/*BRAFV600E* genotype and enrichment in the unfavorable MSS/*BRAFV600E* genotype help explain the poor prognosis of early onset CRC.

Colorectal cancer (CRC) is one of the most common adult malignancies in the United States (US) with a median age at diagnosis of 64 years. It occurs only rarely in young adults and children. Based on population-based data from the Surveillance, Epidemiology, and End Results database, the age-specific incidence of CRC per 100,000 individuals in patients age 25–29 years is 1.6 compared to 241.2 for patients 75 years and older [1]. When CRC occurs in young patients, the prognosis is poor. Reports from several treatment centers around the world have shown that young patients present at a more advanced stage and as a group have a low survival rate [2–9,43,44]. Whereas in the adult population approximately 50% of patients are cured of cancer, in early onset patients, the overall survival rate ranges from 15% to 25% [2,3,5–7,9].

The reasons underlying the poor outcomes of early onset CRC are not well understood. Diagnostic delay because of low suspicion of cancer and failure to work-up symptoms in a timely manner probably accounts for some of the survival difference. However, differences in tumor biology are also important. For example, high grade cancers and signet ring-cell carcinomas are much more common among early onset patients [10,11]. Metastatic spread to regional lymph nodes is common. This suggests early onset CRCs often behave aggressively and may have unique biological features.

There are only a limited number of studies evaluating genetic markers in early onset CRC. In 1991, Dunlop and colleagues [12,13] studied 50 cases of CRC diagnosed before age 30 years and reported that 14 of these patients possessed a mutation in the *MLH1* or *MSH2* mismatch repair gene. In 2000, our group reported clinical and molecular findings in a group of patients with CRC diagnosed at or before age 21 years [14]. In addition to the overall poor prognosis, the striking findings were the high frequency of non-familial cases and enrichment of microsatellite unstable tumors. Although microsatellite instability (MSI) was common, very few cases had classical clinical features of hereditary non-polyposis colorectal cancer (HNPCC) despite the strong prevalence (40%) of MSI.

* Corresponding author at: Yale Surgery—Gastrointestinal/Oncology, PO Box 208062, New Haven, CT 06520-8062, United States. Fax: +1 203 737 4067.
E-mail address: sajid.khan@yale.edu (S.A. Khan).

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To better understand the unique clinical and biologic features of early onset CRC, we assembled a study group of cases with the assistance of the Surgical Committee of the Children's Oncology Group. Archival tumor samples and clinical data were collected for a cohort of patients diagnosed with CRC before the age of 30 years. MSI, *KRAS* codon 12/13 mutations, and *BRAFV600E* mutations were assessed. Clinical presentation, tumor pathology, genetic alterations, and outcomes were compared to a control group of adult onset CRC patients diagnosed after the age of 50 years. The goal of the study was to search for distinguishing genetic features, unique patient subsets, and other clues to explain the poor survival seen in early onset CRC.

1. Materials and methods

1.1. Patient selection

The study comprised two patient groups. The first included 275 male and female patients ≥50 years of age at diagnosis (median = 67; range 50–90) who presented at Memorial Sloan-Kettering Cancer Center (MSKCC) between 1991 and 2005 for surgical treatment of primary colorectal adenocarcinoma with or without synchronous metastases to the liver, lung, peritoneal cavity, or other distant sites. Cases were accrued prospectively to a tissue collection protocol. Tissue was available as frozen, OCT embedded blocks and archival paraffin blocks. The second group included 94 male and female patients diagnosed ≤30 years of age (median = 24.7; range 11–30) treated by colectomy between 1971 and 2005. Availability of paraffin embedded tissue adequate for DNA extraction and immunostaining was required for enrollment. Cases were anonymized and assigned research codes prior to molecular testing and data analysis. Clinical information was collected by chart review at each participating institution. Data documenting type of operation, adjuvant therapy and inflammatory bowel disease were not available. All work was approved by institutional review boards (IRB).

1.2. Review of pathology slides

Hematoxylin and eosin stained sections were reviewed by an expert pathologist (J.S.) and scored as previously described [15].

1.3. Tumor microdissection and DNA extraction

Three to five 10-μm paraffin sections were cut with microtome for tumor and matched normal colonic mucosa. Tumor sections were microdissected to exclude normal mucosa, stroma, and necrotic tissue. For snap frozen tissues, microdissection was guided by a hematoxylin stained section taken from OCT blocks using cryotome. Phenol-based technique was used to extract DNA [16].

1.4. Microsatellite instability analysis and KRAS and BRAF mutation detection

MSI analysis and detection of codon 12/13 *KRAS* mutations and *BRAFV600E* mutations have been described previously [17–19].

1.5. Mismatch repair gene immunohistochemistry (IHC)

Intratumoral expression of *MSH2*, *MLH1*, *MSH6*, and *PMS2* was assessed on 4 μm paraffin sections using established protocol [20].

1.6. Statistics

Analysis of proportions was accomplished by chi-square test, survival displayed by Kaplan–Meier method, and survival differences assessed by log rank test. Stratified test was used to adjust for single covariate. Multivariate Cox regression was used for more adjustments. The analyses were performed using SAS 9.3 (Cary, NC). Significance level was set as $P < 0.05$, two-sided.

2. Results

A comparison of clinical, pathological and molecular features of the two patient groups revealed several clear differences (Table 1). Early onset patients were more likely to present with CRC of advanced TNM stage (76% vs. 50%, $P < 0.0001$). Survival of early onset patients was far worse than for adult patients (Fig. 1a, 5-year disease-specific survival 48% vs 78%, $P < 0.001$). Early onset patients had a higher proportion of poorly differentiated tumors (37% vs. 12%, $P < 0.0001$). This difference was especially notable for signet ring-cell carcinomas (13% vs. <1%, $P < 0.00001$) indicating a large over-representation of this histological subtype in early onset cases.

The other clinical feature distinguishing the early onset group was a higher frequency of a positive family history for CRC (43% vs 26%, $P < 0.10$) (Table 1). However, more than half of early onset patients reported no family history of CRC. Furthermore, very few patients (5%) in the early onset group fulfilled Amsterdam II criteria for HNPCC. In multivariate Cox regression analysis, the hazard ratio of early onset versus adult group is 1.96 (95% CI 1.29 to 2.98, $P = 0.002$) after adjusting for significant survival predictors based on univariate analysis (Table 2).

From genetic analysis we found no difference in the overall prevalence of *BRAFV600E* and *KRAS* codon 12/13 mutations between age groups (Table 1). However, there was greater than a 2-fold increase in the prevalence of MSI tumors in the early onset group (27% vs. 13%, $P < 0.01$). Given the large proportion of MSI tumors in the early onset group, we were interested to know if MSI identifies a subset of patients with unique clinical features. Among the 275 adult onset cases, MSI genotype strongly correlated with clinical characteristics previously associated with MSI biology: right sided tumor location, early stage of disease, high proportion of poorly differentiated cancers, and favorable disease-specific survival (DSS) (Table 2, Fig. 1b) [21,22]. Interestingly, these clinical characteristics were not evident among early onset MSI cancers. Tumor location, tumor grade, and tumor stage at presentation were no different in MSI versus MSS patients in the early onset group (Table 2). In the early onset patients, MSI cancers did have improved survival compared to MSS cancers (Fig. 1c, P = 0.045). However, survival of MSI patients in the early onset group was still far lower than MSI genotype in adult onset cases. In an adjusted Cox model (Table 2), MSI/MSS was a significant predictor independent to age of onset (HR: 0.42; 95% CI 0.22 to 0.83, $P = 0.01$).

To explore potential differences in MSI biology in each age group, we tested all MSI cancers with sufficient archival tumor tissue for intratumoral expression of four MMR genes using IHC. Adult onset MSI cases revealed that loss of MMR gene expression was almost completely

Table 1
Clinical and molecular features of early onset and adult onset colorectal cancer.

Characteristics	Early Onset (N = 94)	Adult Onset (N = 275)	*P*
Median age, years	27	67	–
Sex: male	45 (48)	146 (55)	NS
Family History of Colorectal Cancer	40 (43)	74 (27)	NS
Amsterdam II	5 (5)	2 (<1)	NS
Location: proximal	32 (34)	96 (35)	NS
Stage: III/IV	71 (76)	140 (46)	<0.0001
Histology			
Signet ring-cell	12 (13)	3 (1)	<0.0001
Poorly differentiated	35 (37)	22 (8)	<0.0001
5-year disease-specific survival	48%	78%	<0.0001
MSI	25 (27)	36 (13)	<0.01
BRAFV600E mutation	8 (9)	22 (8)	NS
KRAS codon 12/13 mutation	26 (28)	99 (36)	NS

MSI, microsatellite instability.
Data are expressed as no. (%) unless otherwise noted.