

Molecular Genetic Characteristics of Colorectal Cancer in Patients 90 Years and Older Differ from the Findings in Younger Patients

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ABSTRACT

AIM: Only limited information is available on the molecular characteristics of colorectal cancers diagnosed in patients 90 years and older. There are indirect suggestions that in cancers from very elderly patients, molecular genetic changes may be either more, or less prevalent; thus, raising the question as to the similarity in genetic changes found in colorectal cancers between very elderly and younger patients. We examined several molecular changes associated

with colorectal cancers in 41 very elderly patients, and compared the results to the findings of a younger cohort, between ages 55 and 79 years.

METHODS: We evaluated MSI, loss of heterozygosity for *APC* and *DCC* genes, *KRAS* and *BRAF* gene mutations, and DNA methylation in colon cancer tissue samples using standard PCR techniques.

RESULTS: our data indicate that colorectal cancers from very elderly patients are more frequently right-sided and more likely to demonstrate microsatellite instability. If the cancers contain a *KRAS* mutation, it is less likely to be in the second codon position. Finally, *KRAS* 61 may be more frequent in the very elderly.

CONCLUSIONS: Overall, the colorectal cancers from our very elderly patients, 90 years and older, have at least as many, if not more, molecular genetic changes than the cancers from younger.

Key words: Colorectal cancer; Microsatellite instability; Loss of heterozygosity; *KRAS* gene mutation; Methylation

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INTRODUCTION

Colorectal cancer is the third most commonly diagnosed non-skin cancer and the second (male) or third (female) leading cause of cancer deaths in the United States^[1]. Estimates for new cases for 2017 in the United States are approximately 95,500 for colon cancer and 39,900 for rectal cancer^[1]. Colorectal cancer is most frequently diagnosed in people aged 65-79^[2]. However, 22% of patients with colorectal cancer are 80 years or older at the time of diagnosis. One-third of all deaths from colorectal cancer (27% of men and 40% of women) will occur in individuals aged 80 years and older^[2]. Furthermore, advancing age has been suggested as a potential risk factor for

the development of colorectal cancer^[3].

Colorectal cancers arise, in part, from accumulation of genetic alterations involving one of several major pathways. One is the “adenoma-carcinoma sequence” that primarily influences tumor development in the sigmoid colon and rectum, and involves changes in several genes, e.g. Adenomatous Polyposis Coli (*APC*), Deleted in Colorectal Cancer (*DCC*), Ki-ras2 Kirsten rat sarcoma (*KRAS*) and *p53*^[4]. Other pathways include chromosomal instability, ‘CpG island methylator phenotype’ (CIMP), and the related microsatellite instability (MSI) pathway^[5].

A study by Tomasetti *et al.* suggested that the variation in cancer risk among tissues can be explained by the number of stem cell divisions^[6]. In tissues such as the colon where cell turnover is high and ongoing throughout a person’s life, the frequency of stem cell proliferation will also be high, thus facilitating the accumulation of genetic mutations that are primarily responsible for the development of a cancer; although the authors point out that environmental and hereditary factors may also contribute. Further, based on Tomasetti’s work, one might anticipate more genetic changes in colorectal cancers found in the very elderly, compared to the findings in younger patients. However, the Peto paradox described the observation that cancer incidence does not increase, as theoretically expected, over the human life span^[7]. A more recent study utilizing SEER data concluded that almost all cancers peak at the approximate age of 80 years^[8]. These authors attributed the falling cancer incidence after age 80 to increasing senescence and decreased proliferative potential, and therefore one might anticipate no significant difference in the molecular changes in colorectal cancer between very elderly and younger patients.

The elderly population of the U.S. is increasing rapidly. In 1960, only 9% of the population was age 65 or older, but this demographic group had increased to 15% by 2014. From 2000 to 2010, the cohort of the U.S. population age 85 years and older increased by approximately 30%, an increase of 1,250,000 people^[9]. The elderly are of particular interest, as certain colorectal cancers diagnosed in older age may be associated with poorer prognosis^[10].

A complicating factor is that people of advanced age may not have undergone recent colorectal cancer screening, as based on current recommendations^[11].

There is limited information on the molecular characteristics of colorectal cancers diagnosed at extreme older age, that is, 90 years and older; raising the question whether they are similar or different molecularly from colorectal cancers found in younger patients. The aim of this study was to examine several molecular changes associated with colorectal cancers in these very elderly patients, and to compare the results to the findings of a younger population. We evaluated MSI, loss of heterozygosity (LOH) for *APC* and *DCC* genes, *KRAS* and *BRAF* gene mutations, and DNA methylation in colon cancer tissue samples. Younger control patients were between the ages of 55 and 79 years.

MATERIALS AND METHODS

All samples were archived material from the Department of Pathology, Saint Barnabas Medical Center, Livingston, NJ. Clinical material used in this study primarily represented a suburban community of middle economic level, with a substantial representation from various minority groups (African American and Asian) of both middle and low socio-economic status. We reviewed the computer database of the Department of Pathology for archived cases of primary colorectal cancer surgery in elderly patients of

age 90 years or more. Histological slides stained with H&E stain and paraffin blocks were available for all cases. The same surgical pathologist reviewed all histological slides and indicated the areas for molecular study. Right-sided colorectal cancers included tumors located in the cecum, ascending and transverse colon, whereas left-sided included tumors located in the descending, splenic flexure, sigmoid colon and rectum.

This is a case-case study, as both groups are comprised of cancers. We identified 41 cancer cases in patients 90 years or older. They represented all primary surgical cases of colorectal cancer in this age group identified for the years 1989 through 2015. We chose to limit the older group to patients 90+ years for several reasons. First, if molecular changes in colorectal cancer were dissimilar to those changes in younger patients, it might be most obvious in patients at the extreme of age. Second, existing literature on this topic rarely includes patients 90+ years of age. The younger group consisted of 117 patients between ages 55-79 years who underwent primary resection during the same years as the older group. We restricted the controls to carcinomas lacking residual adenomatous tissue, to minimize sampling issues. The Saint Barnabas Medical Center Institutional Review Board approved the study under a limited data certification for material de-identified of any protected health information.

DNA Extraction and Purification, PCR and Gel Electrophoresis

All tissue specimens were formalin-fixed and paraffin-embedded. Histological slides stained with H&E were examined and the area of relevant tissue was identified and marked. Comparable areas from unstained sections were isolated using a blade and transferred to an Eppendorf tube. Paraffin wax was removed by xylene, followed by ethanol washes. Cellular material was lysed in a proteinase-K buffer solution, and DNA was isolated and purified. DNA was stored at 4°C in 10 mmol/L Tris-EDTA buffer (pH 9.0).

Sequence analysis of the *KRAS* and *BRAF* genes

The codon 12/13 region in exon 2 of the *KRAS* gene was amplified using the primer set 5’-AAGGCCTGCTGAAAATGACTG-3’ and 5’-GGTCCTGCACCAGTAATATGCA-3’. The codon 61 region in exon 3 of the *KRAS* gene was amplified using the primer set 5’CAGACTGTGTTTCTCCCTTCTC-3’ and 5’-CCCTCCCCAGTCCTCATG-3’. The codon 600 region in exon 15 of the *BRAF* gene was amplified using the primer set 5’-CATAATGCTTGCTCTGATAGGAAA-3’ and 5’-GATCCAGACAACCTGTTCAAAGT-3’. Hot-start PCR was performed in 50 µl volumes with AmpliTaq Gold polymerase and ABI reagents (Applied Biosystems, Foster City, CA) using 100 ng of template DNA, 50 pmols of primer, and 2.0 mM MgCl₂ on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). PCR consisted of an initial eight minute denaturation at 94°C, followed by 40 total cycles of a 30 second denaturation at 94°C, a 30 second annealing, and a one minute elongation at 72°C, with a final 30 minute extension at 72°C. The annealing temperature was stepped down at 62°C, 60°C, and 58°C for 5, 15, and 20 cycles, respectively.

The post-PCR products were quality checked by agarose gel and then purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) prior to sequencing. The sequencing reactions were performed in 20 µl volumes using 0.5X BigDye Terminator Cycle Sequencing Reagents (Applied Biosystems, Foster City, CA) with 5.0 pmol of primer (reverse *KRAS*, forward *BRAF*, or reverse *GNAS*) and 1.0 µl of the purified PCR reaction. Reactions were run

on a GeneAmp PCR System 9700 for 25 cycles using two minutes of extension time. The sequencing reaction fragments were cleaned using isopropanol precipitation. Sequencing products were separated by capillary electrophoresis with an ABI 3130 Genetic Analyzer and the data was processed with Sequencing Analysis v5.2 (Applied Biosystems, Foster City, CA) software.

Microsatellite Instability (MSI) Analysis

MSI was detected using the Bethesda panel of markers, which included two mononucleotide markers Bat25 and Bat26, and three dinucleotide markers D2S123, D5S346, and D17S250. All microsatellite primer sets were ordered through the Life Technologies Custom Oligo Synthesis Service (genomicorders@lifetech.com). In all primer sets the forward primer contained a 5' fluorescent label while the reverse primer contained a 5'-GTGCTT tail.

All PCR reactions were performed in 30 µl volume using 100 ng of template with Applied Biosystems reagents and final 1.5 mM MgCl₂ concentrations. Reactions were run on the GeneAmp PCR System 9700 under the following conditions: 5 minute denaturation at 94°C, followed by 35 cycles of a 30 second denaturation at 94°C, 30 second annealing at 55°C, and a 60 second elongation at 72°C, with a final 30 minute extension at 72°C. PCR products were separated by capillary electrophoresis with an ABI 3130 Genetic Analyzer and the data was processed with GeneMapper v4.0 (Applied Biosystems, Foster City, CA) software.

For all MSI studies neoplastic tissue was evaluated simultaneously with normal colonic mucosal tissue from the same patient. Microsatellite instability for a given primer set was defined as a change in the allele pattern, with the appearance of one or more new PCR products relative to those produced by the normal DNA. A tumor was defined as MSI-high if two or more of the five markers had a changed allele pattern, and was referred to as "MSI".

Loss of Heterozygosity of the APC and DCC genes

Loss of Heterozygosity (LOH) of the APC gene was determined by amplification of the CA repeat region within the D5S346 loci with the primer set 5'-ACTCACTCTAGTGATAAATCGGG-3' and 5'-AGCAGATAAGACAAGTATTACTAGTT-3'. Samples that were homozygous for the D5S346 primer set were analyzed using repeats within the D5S1965 and/or D5S492 loci. The primer sets for D5S1965 and D5S492 were 5'-GCATCTAGATCTTGAGACAGTCTTG-3' / 5'-ATGTCTTACTACCTACATGTTCATGG-3' and 5'-TTTCCCCAATACAACGTGA-3' / 5'-TAGATTTGAATCTTTCCAGTGA-3', respectively.

LOH of the DCC gene was determined by amplification of the CA repeat markers within the D18S58 or D18S61 loci. The primers for D18S58 were: 50 -GCT CCC GGC TGG TTT T-30 (sense) and 50 -GCA GGA AAT CGC AGG AAC TT-30 (antisense) and for D18S61 were: 50 -ATT TCT AAG AGG ACT CCC AAA CT-30 (sense) and 50 -ATA TTT TGA AAC TCA GGA GCA T-30 (antisense). These primers generated PCR products 144 to 164 base pairs and 152 to 184 base pairs, respectively.

PCR primers and reactions were prepared as described in the MSI analysis section. Neoplastic tissue was evaluated simultaneously with normal colonic mucosal tissue from the same patient. To determine LOH, the ratio of the allele band intensities (peak heights) of the neoplastic tissue was divided by the corresponding ratio for the normal mucosa. LOH was defined as a resultant ratio of less than or equal to 0.5.

Methylation Analysis

The methylation status of the mismatch repair (MMR) system was ascertained using the SALSA® MS-MLPA® Methylation-specific DNA detection Kit #ME011 (MRC-Holland, Amsterdam, The Netherlands). This kit uses 22 probes containing a HhaI recognition site to detect the aberrant methylation in seven MMR genes including 5 MLH1 probes, 4 MSH2 probes, 2 MSH6 probes, 1 MSH3 probes, 1 MLH3 probe, 3 PMS2 probes and 6 MGMT probes. An additional 16 reference probes were included that did not have a HhaI site.

Briefly, 200 ng of genomic DNA was hybridized overnight with the 38-probe mix. This hybridization mixture was split for two separate reactions. The first reaction ligated the probes that were hybridized at the potential methylation sites of the genes listed above. Ligation enabled the probes to be subsequently PCR amplified. The second reaction was a dual ligation and HhaI restriction enzyme cutting reaction that ligated the probes and also cleaved the probes at the HhaI site, unless the site was methylated. All of the probes contained universal PCR primer recognition sites. A single PCR reaction could therefore amplify all of the ligation products in both the ligation reaction and the ligation/HhaI reaction (if uncut because the site was methylated).

Electrophoresis of PCR fragments was performed on an ABI 3130 Genetic Analyzer and the raw data was processed with Genemapper v4.0 software. The Genemapper raw data was subsequently exported and the methylation status was analyzed using Coffalyser v9.4 software available at the MRC-Holland website (www.mlpa.com). Within the Coffalyser software, the "Direct methylation Status" analysis option was chosen to normalize and analyze the MS-MLPA data. The methylation status for the 22 probe target sites in each sample was determined by comparing the PCR products from the normal DNA to that of the tumor DNA. In the normal DNA, all probes that cover unmethylated HhaI sites were cut and no PCR product was observed for those probes. Any HhaI probes that hybridized to a methylated site were uncut and subsequently showed a PCR product in the tumor specimen. Ratios of tumor to normal peak areas for a given probe that were in the 0.7 to 1.0 range were assigned as unmethylated. Ratios of tumor to normal peak areas for a given probe that were < 0.3 range are assigned methylated. Ratios in the 0.3 to 0.7 range were considered as partially methylated or hemimethylated.

Statistical Analysis

The Chi square method was used to compare the percent of older CRC cases with particular molecular characteristics to that of the younger CRC group. A p-value of <0.05 was considered as level of significance. The nonparametric Wilcoxon test was used to compare size.

RESULTS

There were 41 very elderly patients 90 years or older, and 117 younger patients between 55 and 79 years in the control group. Thirty-eight of the elderly were between 90 and 94 years of age at the time of surgery, two were 95 and one was 101 years old. Thirty-four of the elderly had a colorectal cancer, while seven of the elderly had a colorectal cancer with residual adenoma present in the resected specimen. Sixteen of the 41 (39%) elderly patients had a simultaneous adenoma at the time of surgery. Five patients had one adenoma, four patients had two, three patients had three, and four patients had four to seven adenomas. We limited the controls to patients with no more than one adenoma found at the time of surgery,

to avoid including anyone with an unrecognized polyposis syndrome. Ninety-two controls had no adenomas and 25 (21.4%) had one adenoma.

Details as to gender and the tumor features of location, size, mucin status, histological grade and stage are given in Table 1. The percentage of female patients was only slightly greater for the elderly, than for the controls, but was not statistically different. There was no difference between the elderly and the controls with regard to right vs. left location of the tumor. However, 37.6% of the controls had a sigmoid cancer, while only 17.1% of the cancers in the elderly were sigmoid in location. There was no difference between the elderly and the controls with regard to the presence of mucin in the tumor or pathological tumor stage. However, there was a wide difference between the two groups with regard to tumor grade. The elderly were more evenly divided by the three grades, while the controls were primarily grade 2. Tumor size was not available for all tumors, but for those cases with recorded gross measurements, the cancers of the elderly were statistically larger than those of the controls and showed greater variation in size. Twelve of the tumors from the elderly were uninformative with regard to LOH because of MSI, and 16 tumors were similarly uninformative in the control group. MSI in a tumor results in the presence of additional alleles that affects the relative quantity of the germline alleles, thus making an LOH comparison unreliable. For the informative cases, there was no difference in the incidence of LOH for *APC* or *DCC*, or in the incidence of *KRAS* mutation, between the two groups (Table 2). The *KRAS* mutations of the tumors from the elderly included: 10 (83.3%) mutations in codon 12: 5 in the first position and 5 in the second position. There were also 2 (16.7%) mutations in codon 13: 1 in the first position and 1

in the second position. The *KRAS* mutations of the tumors from the controls included: 29 (85.3%) mutations in codon 12: 5 mutations in the first position and 24 mutations in the second position). There were also 4 (11.8%) with mutations in codon 13: c.38G>A=4 (second position), and 1 mutation at the third position of codon 19 (Table 3). Thus, for the elderly, 50% (six of twelve) of the *KRAS* mutations involved the second position, while for the controls, 28 of 33 (84.8%) codon 12 and 13 mutations were in the second position. This was a significant difference, with $p = 0.016$, O.R.= 0.178 (0.04-0.78). The majority of the changes involved guanine to adenine for the elderly, but guanine to thymine for the controls. The elderly cases were also assayed for *KRAS* 61, and 3 (7.3%) additional cases were mutated. A *BRAF* mutation was detected in 13 (31.7%) of the elderly cases. Eleven of the 13 elderly cases with a *BRAF* mutation were also microsatellite unstable, and 2 were microsatellite stable (data not shown).

Microsatellite instability was statistically more common in the tumors of the elderly than in the control cases ($p = 0.0078$) (Table 2). This was also true, when comparing just right-sided cancers: 12 of 27 (44.4%) were MSI in the elderly, and 15 of 65 (23%) in controls ($p = 0.04$). All elderly cases with MSI demonstrated methylation; 13 had methylation of *MLH1* and 1 had methylation of *MGMT* (data not shown).

Table 1 Clinical characteristics of patients and their tumors.

	Cases	Controls	O.R.	p-value
	N (%)	N (%)		
Number	41	117		
Age at diagnosis (+/- S.D.)	92.2 (1.9)	69.4 (6.9)		
Gender				
Male	14 (34.1)	54 (46.1)	0.60 (0.29-1.27)	0.18
Female	27 (65.9)	63 (53.8)		
Location				
Right	27 (65.9)	65 (55.6)	1.54 (0.74-3.23)	0.25
Left	14 (34.1)	52 (44.4)		
Mucin Status				
Present	8 (19.5)	19 (16.2)	1.30 (0.52-3.27)	0.57
Absent	31(75.6)	96 (82.1)		
Not known	2 (4.9)	2 (1.7)		
Histological grade				
1	16 (39.0)	23 (19.7)	0.004	
2	10 (24.4)	62 (53.0)		
3	13 (31.7)	28 (23.9)		
Not known	2 (4.9)	4 (3.4)		
TNM stage				
1	8 (19.5)	22 (18.8)	0.52	
2	12 (29.3)	28 (23.9)		
3	14 (34.1)	61 (52.1)		
4	1 (2.4)	5 (4.3)		
Not known	6 (14.6)	1 (0.9)		
Tumor size in cm ³ (mean)	49.5 (n=28)	42.0 (n=97)		0.017

Table 2 Molecular genetic findings in tumors.

	Cases	Controls	O.R.	p-value
	N=41	N=117		
	N (%)	N (%)		
APC gene*				
LOH	9 (33.3)	44 (43.6)	0.65 (0.27-1.58)	0.34
Wild type	18 (66.7)	57 (56.4)		
DCC gene†				
LOH	14 (53.8)	72 (72.7)	0.44 (0.18-1.06)	0.66
Wild type	12 (46.1)	27 (27.3)		
KRAS gene (codons12-13)				
Mutated	12 (29.3)	33 (28.4)	1.01 (0.46-2.21)	0.98
Wild	29 (70.7)	83 (71.6)		
Microsatellite stability‡				
MSI high	14 (34.1)	17 (14.8)	2.99 (1.31-6.83)	0.008
MSS	27 (65.9)	98 (85.2)		

* 1 cancer was not studied and 1 was homozygous for markers used for the elderly, and 12 elderly and 16 controls were MSI and uninformative.

† 3 cancers were not studied and 1 was homozygous for markers used for the elderly, and 2 cancers were not studied for the controls.

‡ 2 cancers were not studied for the controls.

Table 3 Specific *KRAS* mutations in cases and controls.

		Cases (N = 12)	Controls (N = 34)
	Nucleotide	N (%)	N (%)
Codon12	c.35G > T	0 (0)	15 (44.1)
	c.35G > A	5 (41.7)	7 (20.6)
	c.34G > C	1 (8.3)	1 (2.9)
	c.34G > T	3 (25)	2 (5.9)
Codon 13	c.34G > A	1 (8.3)	2 (5.9)
	c.35G > C	0 (0)	2 (5.9)
	c.38G > A	1 (8.3)	4 (11.8)
Codon 19	c.37G > A	1 (8.3)	0 (0)
	c.57G > T	0 (0)	1 (2.9)

DISCUSSION

There are few studies regarding clinical and molecular genetic changes in very elderly patients. McLeary *et al* reported a higher percentage of MSI-high and CIMP-high tumors in an age group above 60 years compared to a younger age group, but no difference between the age groups 60-74 and > 75 years^[10]. Each of their three groups comprised several hundred cases. In their study, no significant difference was found in the prevalence rates of *KRAS*, *BRAF*, and *PIK3CA* mutations. However, the authors did not indicate findings specific to the very elderly.

In the general population, colorectal cancer is more common in men than in women^[2]. However, a tumor registry study by Patel *et al.* reported that elderly patients with colorectal cancer were more commonly female (60%)^[12]. We also found a higher percentage of the very elderly cases to be female, compared to the control group, but because of sample size, this did not reach statistical significance. There are several possible explanations for this change. First, females are much more likely to be living into their 90s than are males. The U.S. Census Bureau data for 2010 indicated a female to male ratio of 2.63: 1 for individuals 90 years or older. Second, studies such as ours, that are based on primary colorectal surgery have unwittingly excluded cases clinically too ill or frail to undergo primary surgery, and that may reflect more males than females.

Arai *et al.* reported that in an elderly group of Japanese patients (age > 85 years), cancers located in the colon proximal to the splenic flexure were significantly more common than those in their younger groups^[13]. A similar finding was reported in Patel's study from California, where patients 80 years or older were more likely to have ascending tumors (55%) when compared to younger groups^[12]. Almost two-thirds of our very elderly cases had right-sided tumors. This contrasts to an aggregate national percentage for patients of all ages of just 41%^[2].

A recent study by Cha *et al.* compared colonoscopic findings of 'extremely elderly' patients (≥ 90 years old) versus 'very elderly patients' (75 to 79-year-old)^[3]. Their 'extremely elderly' group had significantly larger tumors as compared to the very elderly group. In contrast, the study by Patel *et al.* reported the frequency of large tumors (>5 cm) in patients over 80 years to be similar to the frequency found in patients 50-64 and 65 to 79 years^[12]. The tumors from our very elderly patients were statistically larger than the tumors from our controls ($p = 0.017$). This may reflect the predominance of right-sided tumors, which may tend to become clinically apparent later in the disease course than left-sided tumors. A recent study of 1223 cases of colorectal cancer found tumor diameter to be greater for right-sided tumors compared to left-sided tumors^[14]. Also, it is possible that our very elderly cases had not undergone colon screening for many years, thereby permitting further tumor growth. Both prior reports on the very elderly found stage II to be the most common stage, as was true for our controls^[12-13]. However, the tumors from our very elderly group were slightly more likely to be stage III.

LOH of *APC* gene is reported in 30 to 40% of colorectal cancers^[15], similar to our findings for both cases and controls. Similarly, we found no difference in LOH of *DCC* gene or *KRAS* mutation frequency between our cases and controls; both are similar to previously reported findings^[16-17]. Of note, is our finding of a *KRAS* codon 61 gene mutation in 3 (7.3%) of 41 of our cases. A recent large study found only 19 (3.7%) of 513 colorectal cancers with a *KRAS* 61 mutation^[18]. Further study of larger numbers of colorectal cancer in the very elderly would be needed to determine whether a *KRAS*

codon 61 mutation is a more common feature of cancer in this age group. There was a marked difference between our cases and controls with regard to the position of the *KRAS* mutation, with the cases demonstrating relatively more *KRAS* mutations in the first position. Differing *KRAS* mutations may suggest different biochemical pathways involved in the carcinogenetic process^[19].

MSI is a marker for loss of DNA mismatch repair activity, resulting in altered lengths of short repetitive nucleotide sequences. Biallelic methylation of the promotor CpG areas of the mismatch repair gene *MLH1* results in loss of mismatch repair in colorectal cancer, and it is primarily associated with MSI seen in colorectal cancer. The underlying mechanism responsible for the aberrant methylation seen in colorectal cancer is not fully understood. Aging, alone, is associated with methylation of cancer-relevant genes in normal colon mucosa, without evidence of disease pathology^[20]. However, the presence of an accompanying *BRAF* mutation and MSI strongly suggests the pathological 'MSI pathway'. MSI is present in approximately 15% of all sporadic colorectal cancers^[21]; and MSI was identified in 15% of 300 cancers from patients 75 years and older in one study^[12], and in 20.9% of 162 cancers from patients greater than 80 years of age in another study^[22]. We found MSI to be more frequent in our very elderly patients, than in the younger group, even when controlling for right-sided location, with almost half of the right-sided cancers from the very elderly demonstrating MSI. All our very elderly patients with cancers demonstrating MSI were methylated, and 10 of the 12 also were *BRAF* mutated, indicating these tumors followed the pathway of acquired methylation of *MLH1* and the development of microsatellite instability.

Our study has several limitations. We were not able to evaluate the colorectal cancers from the younger patients for the molecular genetic changes of *KRAS* 61, *BRAF*, or methylation. However, in other respects, our younger patients' findings were similar to general literature results. Secondly, there are numerous other molecular genetic changes known to occur in colorectal cancers that we did not study. It is possible that qualitative as well as quantitative differences could exist between the average-aged patient with colorectal cancer and the very old with regard to these other changes.

CONCLUSIONS

In conclusion, our data indicate that colorectal cancer from very elderly patients 90 years and older differ from those cancers from younger patients in several respects. The cancers are more frequently right-sided and more likely to demonstrate microsatellite instability. If the cancers contain a *KRAS* mutation, it is less likely to be in the second codon position. Finally, *KRAS* 61 may be more frequent in the very elderly. Overall, the colorectal cancers from our very elderly patients, 90 years and older, have at least as many, if not more, molecular genetic changes than the cancers from younger aged patients.

DECLARATIONS

1. Ethics approval and consent to participate: The Saint Barnabas Medical Center Institutional Review Board approved the study under a limited data certification for material de-identified of any protected health information.

2. Consent for publication: not applicable.

3. Availability of data and material: The dataset used during the current study is available from the corresponding author on reasonable request.

4. Competing interests: All authors declare that they have no

competing interests.

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6. Authors' contributions: PZ designed the study, interpreted the data and was a major contributor to writing the manuscript. RK contributed to analysis and manuscript preparation. SM performed the molecular analyses and contributed to manuscript preparation. MS-S contributed to specimen analyses and manuscript preparation.

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