Molecular characterization of colorectal tumors in young patients compared with older patients and impact on outcome

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BACKGROUND

- Colorectal cancer (CRC) is increasingly diagnosed in adults <50 years old, often at an advanced stage and with a worse prognosis.
- Limited data suggests tumors that develop in a younger cohort show distinct genetic changes that are different from classic CRC in older adults. It is unclear how these differences effect clinical outcomes.

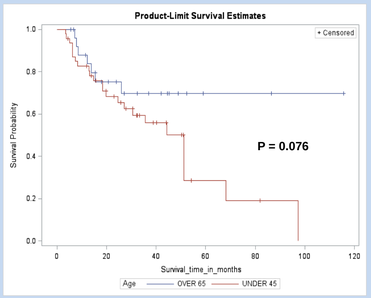
AIM: To compare profiles of genetic alterations and clinical variables between younger and older patients to further elucidate differences and their impact on survival.

METHODS

- Molecular profiles of 4,821 tumors from young (≤ 45 years; n=1,277) and old (≥ 65 years; n=3,544) CRC patients were obtained from Caris Life Sciences. Protein expression (IHC), gene amplification (ISH), sequencing (NGS and Sanger), and fragment analysis were performed to generate profiles.
- Fisher's exact two-tailed tests were used to determine molecular differences between the two age groups.
- CRC cases from 2005 to 2015 at the Lombardi Comprehensive Cancer Center with associated Caris Life Sciences tumor molecular profiles were analyzed to identify young (≤ 45 years) and old (≥ 65 years) patient cohorts for the clinical outcome correlation portion of study. Forty-seven patients ≤ 45 years old and twenty-seven patients ≥ 65 years old were identified.
- Retrospective review was completed on these seventy-four patients to determine clinicopathologic features including sex, race, stage at diagnosis, tumor differentiation, CEA level at presentation, date of diagnosis, and survival status.
- Kaplan-Meier methodology was used to estimate survival outcomes between the two age groups. Fisher's exact two-tailed tests were used to determine clinicopathologic differences between the two age groups.

RESULTS

Figure 1. Overall Survival, ≤ 45 years old at diagnosis compared to ≥ 65 years old



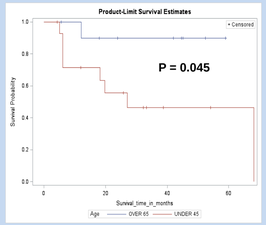
- Median overall survival (OS) in the younger cohort was 51.1 months versus not reached (NR) in the older cohort (p=0.076).
- As seen in Table 4, a significantly higher number of younger patients were metastatic at time of diagnosis.

Table 1. Impact of biomarker expression or genetic mutation on overall survival

Variable	<= 45 (p value)	>=65 (p value)	Age groups compared	
			positive	*negative*
IHC TS	0.763	0.521	0.225	0.274
IHC ERCC1*	0.269	0.137	0.556	***0.045****
IHC TOPO1	0.853	0.128	0.684	0.100
IHC PD1	0.764	0.060	0.097	0.882
SEQ APC	0.376	0.233	0.202	0.321
SEQ KRAS	0.619	0.723	0.382	0.087
SEQ SMAD4	0.287	n/a	n/a	0.221

- No statistically significant differences in overall survival were noted with biomarker expression or mutated gene status within each age group.
- Evaluating biomarker expression positivity or mutated gene and impact on survival between age groups, no statistically significant differences were found.
- Evaluating lack of biomarker expression or mutated gene and impact on survival between age groups, ERCC1 underexpression was associated with lower overall survival in the younger cohort (p=0.045).

Figure 2. Overall survival stratified by age group in ERCC1 negative patients



RESULTS

Table 2. Frequency of selected biomarker positivity and genetic mutations in clinical cohort

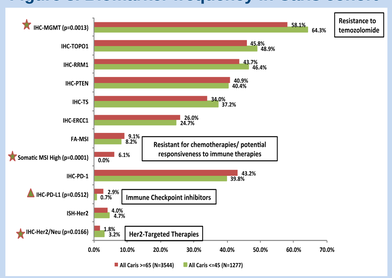
Variable	<=45 (n=47)	>=65 (n=27)	P-value
IHC TS	48%	56%	0.612
IHC ERCC1	17%	31%	0.429
IHC TOPO1	56%	56%	1.000
IHC PD1	32%	50%	0.337
IHC PD-L1	4%	0%	1.000
IHC Her2	5%	4%	1.000
ISH Her2	6%	11%	0.602
IHC MGMT	n/a	n/a	n/a
SEQ BRCA1	0%	0%	n/a
SEQ BRCA2	9%	8%	1.000
SEQ APC	56%	45%	0.585
SEQ KRAS	59%	46%	0.318
SEQ BRAF	5%	8%	0.644
SEQ SMAD4	**34%**	**0%**	***0.002****
SEQ KDR	6%	0%	0.508
SEQ VHL	0%	0%	n/a

Table 3. Frequency of selected biomarker positivity and genetic mutations in Caris cohort

Selected Biomarkers Tested	<=45 (N=1277)		>=65 (N=3544)		p value	Potential therapy implications
	Positive N / Total N	Percent	Positive N / Total N	Percent		
FA-MSI High	16/194	8.2%	42/462	9.1%	ns	Some resistance to chemotherapy/potential responsiveness to immune therapies
Somatic MSI High	**0/187**	**0.0%**	**27/440**	**6.1%**	***0.0001****	
IHC-ERCC1	144/582	24.7%	365/1406	26.0%	ns	Resistance to platinum agents
IHC-Her2/Neu	**30/940**	**3.2%**	**40/2248**	**1.8%**	***0.0166****	Sensitivity to Her2-Targeted Therapies
ISH-Her2	25/532	4.7%	48/1186	4.0%	ns	
IHC-MGMT	**606/943**	**64.3%**	**1304/2243**	**58.1%**	***0.0013****	Resistance to temozolomide
IHC-PD-1	113/284	39.8%	282/653	43.2%	ns	
IHC-PD-L1	2/287	0.7%	19/661	2.9%	0.0512	
IHC-PTEN	423/1048	40.4%	1049/2567	40.9%	ns	
IHC-TOPO1	481/983	48.9%	1126/2458	45.8%	ns	Sensitivity to Irinotecan
IHC-TS	370/994	37.2%	843/2477	34.0%	ns	Resistance to fluoropyrimidines
SEQ-TP53	254/407	62.4%	617/1026	60.1%	ns	
SEQ-APC	**220/408**	**53.9%**	**647/1036**	**62.5%**	***0.0034****	
SEQ-KRAS	**444/1073**	**41.4%**	**1387/3058**	**45.4%**	***0.0245****	Resistance to EGFR Monoclonal Abs
SEQ-PIK3CA	75/507	14.8%	212/1262	16.8%	ns	Sensitivity to PI3K/Akt/mTor inhibitors
SEQ-SMAD4	60/406	14.8%	127/1026	12.4%	ns	
SEQ-BRCA2	28/226	12.4%	27/236	11.4%	ns	Sensitivity to Platinum/PARP inhibitors
SEQ-BRCA1	18/226	8.0%	13/236	5.5%	ns	
SEQ-HNF1A	24/356	6.7%	67/915	7.3%	ns	
SEQ-FBXW7	22/403	5.5%	98/1021	9.6%	ns	
SEQ-BRAF	**46/951**	**4.8%**	**361/2511**	**14.4%**	***<0.0001****	Combination therapies including a BRAF inhibitor
SEQ-NRAS	19/490	3.9%	56/1258	4.5%	ns	

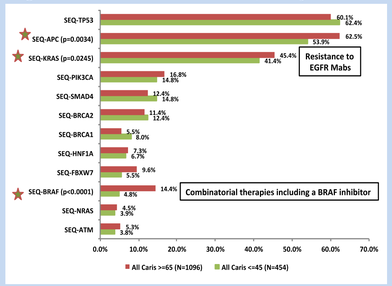
Highlighted cells are biomarkers with frequencies significantly higher than the other age group as tested by Fisher's exact two-tailed tests

Figure 3. Biomarker frequency in Caris cohort



- Selected biomarker frequencies tested by IHC or fragment analysis observed in the Caris cohort.
- Somatic MSI was determined by concurrent BRAF mutation with MSI high by fragment analysis.
- Therapeutic agents in boxes are associated with the corresponding biomarker aberrations.
- Stars indicate biomarkers with frequencies significantly different from the other age group as tested by Fisher's exact two-tailed tests; triangle shows trend.

Figure 4. Genetic mutation frequency in Caris cohort



- Twelve genes with the highest mutation rates with NextGen sequencing taken from patients younger than 45 (n=454) or older than 65 years (n=1096).
- Therapeutic agents in boxes are associated with the corresponding biomarker aberrations.
- Stars indicate biomarkers with frequencies significantly different from the other age group as tested by Fisher's exact two-tailed tests.

Table 4. Comparison of select clinicopathologic features between young and older cohort

Clinical Variable	<=45 (n=47)	>=65 (n=27)	P-value
Sex			
Male	36%	41%	0.805
Female	64%	59%	
Race			
Caucasian	69%	69%	0.800
African American	19%	19%	
Other	12%	12%	
CEA level at diagnosis	27.7	16.1	0.748
Primary site of disease			
Right Colon	34%	33%	0.848
Transverse Colon	17%	11%	
Left Colon	49%	56%	
Stage at diagnosis			
I	2%	7%	0.073
II	4%	15%	
III	26%	37%	
IV	68%	41%	
Metastatic disease at diagnosis	79%	44%	***0.005****
Degree of tumor differentiation			
Well	12%	20%	***0.041****
Moderate	76%	56%	
Poor	5%	24%	
Microsatellite instability	3%	13%	0.196
Underwent surgical resection	89%	89%	1.000
Survival Status			
Alive	49%	74%	***0.05****
Deceased	51%	26%	

- Most frequently mutated genes included TP53, APC, KRAS, PIK3CA, SMAD4, and BRCA1/2.
- Mutation rates for BRAF ($p < 0.0001$), APC ($p=0.0034$), and KRAS ($p=0.025$) were higher in older patients.
- NRAS mutation rates were similar in both groups.
- Younger patients had higher overexpression rates of HER-2/neu ($p=0.017$) and MGMT ($p=0.001$).
- There was no difference in TS, ERCC1, or TOPO1 expression between age groups.
- Microsatellite instability (MSI) was similar between cohorts (10.3% vs. 8.1%), but somatic MSI high (determined by concurrent BRAF mutation) was higher in older patients (6% vs. 0%, $p < 0.0001$).
- In the clinical cohort, SMAD4 mutation was more common in younger patients ($p=0.002$). Other mutations and biomarker expression levels were not significantly different between age groups.

CONCLUSIONS

- Younger CRC patients were more likely to present with metastatic disease and had a trend toward lower overall survival.
- There were no significant differences in sex, race, or primary site of disease between age groups.
- Microsatellite instability occurs at similar frequencies in the young and older cohorts. Interestingly, somatic MSI high was seen exclusively in older patients.
- Older patients had higher rates of BRAF, APC, and KRAS mutations, whereas younger patients had higher overexpression of HER-2/neu and MGMT and an increased number of SMAD4 mutants.
- Younger patients without significant ERCC1 expression experienced lower overall survival as compared with the older cohort. No additional differences in overall survival based on biomarker expression or mutation status in patients with clinical outcome data were revealed.
- Our findings suggest there are distinct genetic differences in younger patients as compared to older patients with CRC. In our limited clinical cohort, however, these genetic differences did not appear to impact survival.
- Continued efforts are needed to further understand the significance of these differences to allow for the development of tailored screening and treatment strategies for both age groups of CRC patients.