Therapeutic biomarker differences between MSI-H and MSS colorectal cancers

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Abstract #3597

Background: Approximately 15% of colorectal cancers (CRC) display high level of microsatellite instability (MSI-H) due to either hereditary predisposition (Lynch syndrome, LS) or somatic hypermethylation of MLH1. They carry a significantly different prognosis and responses to treatments compared with microsatellite stable (MSS) or low microsatellite instability (MSI-L) CRC. We investigated therapeutically important biomarkers, which may underlie different treatment options for CRC.
Methods: Sixty-four MSI-H (including 20 confirmed LS cases), 9 MSI-L and 558 MSS cases were profiled at Caris Life Sciences (Phoenix, AZ) using immunohistochemistry and sequencing (NextGen and Sanger).
Results: Compared with non-MSI-H, MSI-H tumors had significantly higher expression of Thymidylate Synthase (TS) (85% vs. 31%), PTEN (71% vs. 48%) expressions and significantly higher mutation rates of *BRAF* (35% vs. 5%), *CTNNB1* (10% vs. 0.7%), *HNF1A* (32% vs. 0.2%), *BRCA1* (19% vs. 5%) and *BRCA2* (50% vs. 14%). MSI-H cancers were also significantly more often infiltrated with PD-1+ lymphocytes (71% vs. 43%). Features found specific to sporadic MSI-H tumors (defined as MSI-H and *BRAF V600E*) in comparison with non-MSI-H tumors included higher mutation rates on select genes within the PI3K/AKT/mTOR pathway, including *FBXW7* (31% vs. 7%), *PTEN* (19% vs. 3%) and *STK11* (18% vs. 1%). 20 confirmed LS cases also exhibited significantly higher TS (100%) expression, *CTNNB1* (10%) and *HNF1A* (40%) mutations than non-MSI-H and lower *FBXW7* (10% vs. 31%), *PTEN* (10% vs. 19%) and *STK11* (0% vs. 18%) mutation rates than sporadic MSI-H tumors (all $p < 0.02$).
Conclusions: Significantly higher TS expression is a characteristic of both sporadic MSI-H and Lynch tumors, potentially explaining the observed reduced clinical benefit from 5-FU. Higher PD-1+ TIL, *BRCA1/2* and *CTNNB1* mutations suggest MSI-H as a more promising group for targeted immunotherapy, PARP and Wnt pathway inhibitors. Different molecular features of sporadic MSI-H and Lynch subgroups including PI3K/AKT/mTOR offer insight into targeted therapies for these subgroups of CRC.

Background

Colorectal cancer (CRC) represents the third most common malignancy in the Western population. Recent advances in molecular research have resulted in new insights into the molecular and genetic features of CRC. Among new discriminating markers in CRC, microsatellite instability (MSI) appears to be one of the most relevant (Thibodeau 1993).
Approximately 15% of CRC display high levels of MSI (MSI-H) , with about 12% caused by sporadic acquisition of hympermethylation of the MLH1 gene promoter, usually associated with CpG island methylator phenotype (CIMP) and only 3% associated with Lynch syndrome. MSI-H tumors carry a significantly different prognosis and response to treatments compared with microsatellite stable (MSS) or low MSI (MSI-L) CRC (Hong 2012; Guetz 2009). In the present study, we investigated therapeutically important biomarkers, which may underlie different treatment options for CRC.

Methods

The study included 64 MSI-H (including 20 confirmed LS cases), 9 MSI-L and 558 MSS cases that were profiled at Caris Life Sciences (Phoenix, AZ) using immunohistochemistry (IHC) and sequencing (NextGen and Sanger).
MSI-H status was determined using a combination of IHC (MLH1, PMS2, MSH2, MSH6) and MIA (Microsatellite Instability Analysis) fragment analysis. MIA included fluorescently labeled primers for co-amplification of seven markers including five mononucleotide repeat markers (BAT-25, BAT26, NR-21, NR24 and MONO-27) and two pentanucleotide repeat markers (Penta C and D). The mononucleotide markers were used for MSI determination while the pentanucleotide markers were used to detect either sample mix-ups or contamination. A sample was considered MSI-H if two or more mononucleotide repeats were abnormal while MSI-L if one mononucleotide repeat was abnormal. The tumors were considered MSS if mononucleotide repeats were identical between the tumor and adjacent normal tissue.
Other specific tests were performed per physician request. Biomarker associations were calculated by two-tailed Fisher Exact tests.

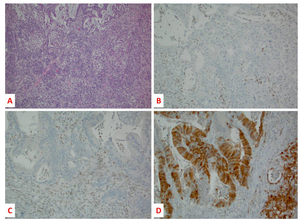
Results

Table 1: Patient Characteristics

	Total N	Average Age	Male Percent	
Complete cohort	**629**	**59 yrs**	**49.0%**	
MSI-H cohort	**64**	**59 yrs**	**50.0%**	
BRAF-mutated MSI cohort	17	73 yrs	24.0%	Sporadic MSI
BRAF-wildtype MSI-H cohort	23	55 yrs	65.0%	
Confirmed Lynch cohort	20	47 yrs	61.1%	Lynch syndrome
MSI-Negative cohort	**567**	**59 yrs**	**49.0%**	

- Consistent with previous studies, in our cohort, MSI-H patients with sporadic CRC are older in age and are more likely to be female.
- On the other hand, patients with Lynch syndrome are significantly younger in age and are more likely to be male.
- In general, patients with MSI-H phenotype have similar ages and gender compared to those that are MSI-Negative.

Figure 1: Sporadic MSI-H CRC (BRAF V600E) with a poor differentiation and tumor-infiltrating lymphocytes (**A**), due to the loss of MLH-1 (**B**) and its interacting partner PMS2 (**C**) proteins. Strong overexpression of TS (2+ in 80% cancer cells - **D**)



Results

Figure 2: Biomarker features in molecular subgroups of CRC tumors: Features that are common to MSI-H tumors and that are specific to sporadic MSI-H tumors are identified. Blue: higher frequency; red: lower frequency; N/A: test not performed.

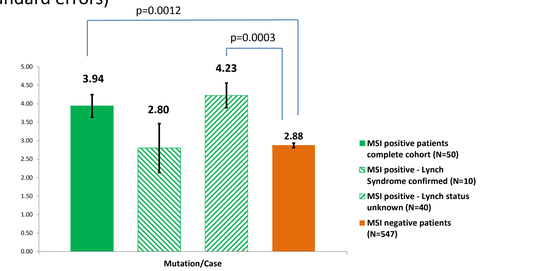
	MSI-High (n=64): BRAF mutated (N=17)	MSI-High (n=64): BRAF wild type (N=23)	MSI-High (n=64): Confirmed Lynch Syndrome (N=20)	MSI-Negative (N=567: 9 MSL-L; 558 MSS)	Cancer pathway /Therapeutic approach
TS_IHC	88% (15/17)	78% (18/23)	100% (19/19)	31% (169/550)	DNA synthesis
		85% (52/61)			
RRM1_IHC	76% (13/17)	70% (16/23%)	N/A	44% (240/549)	
		71% (30/42)			
PTEN_IHC	82% (14/17)	70% (16/23)	N/A	48% (267/552)	
		71% (31/42)			
HNF1A_SEQ	31% (5/16)	29% (6/21)	40% (4/10)	0.2% (1/461)	
		32%(15/47)			
BRCA1_SEQ	29% (2/7)	16% (3/19)	N/A	5.5% (21/383)	Homologous Recombination
		14% (5/36)			
BRCA2_SEQ	43% (3/7)	53% (10/19)	N/A	14% (53/381)	
		36% (13/36)			
PD1_IHC	82% (14/17)	65% (15/23)	69% (9/13)	43% (237/551)	
		71% (33/55)			
SMO_SEQ	7.7% (1/13)	5.6% (1/18)	10% (1/10)	0.2% (1/448)	
		7.3% (3/41)			
CTNNB1_SEQ	6% (1/17)	13% (3/23)	10% (1/10)	0.7% (4/537)	Wnt pathway
		10% (5/50)			
APC_SEQ	35% (6/17)	36% (8/22)	50% (5/10)	60% (329/541)	
		39% (19/49)			
TUBB3_IHC	0% (0/17)	17% (4/23)	N/A	37% (202/549)	
		12% (5/42)			
TP53_SEQ	38% (6/16)	26% (6/23)	40% (4/10)	63% (338/533)	
		33% (16/49)			
NRAS_SEQ	0 (0/17)	0 (0/22)	0 (0/10)	6.2% (33/533)	MAPK pathway
		0 (0/49)			
KRAS_SEQ	5.9% (1/17)	57% (13/23)	50% (5/10)	54% (249/539)	
PTEN_SEQ	19% (3/16)	4.5% (1/22)	10% (1/10)	3% (15/503)	PI3K/Akt/mTOR inhibitors
STK11_SEQ	18% (3/17)	0% (0/21)	0% (0/10)	1.4% (7/506)	
FBXW7_SEQ	31% (5/16)	14% (3/22)	10% (1/10)	7% (37/529)	

Sporadic (CIMP) — Lynch syndrome CRC

- High expression of TS in both sporadic MSI-H and Lynch tumors suggests lack of benefit from fluorouracil in sporadic MSI-H CRC patients, very similar to that seen in patients with Lynch syndrome. In CRC patients who are MSI-negative, TS expression is significantly lower, underlying clinical benefit seen in majority of CRC patients.
- Homologous Recombination Deficiency is higher in MSI-H patients, suggesting increased response to PARP-inhibitors and DNA-damaging agents.
- As shown by mutations in PTEN, STK11 and FBXW7, PIK3CA/Akt/mTor pathway activation is high in sporadic MSI-H CRC but low in Lynch tumors and MSI-low tumors, suggesting therapeutic opportunities for sporadic MSI-H CRC.

Results

Figure 3: Mutations per case in subgroups of CRC tumors (error bars show standard errors)



- The 47-gene mutation panel shows that MSI-H tumors (n=50) carry more mutations per case than MSI-negative tumors (n=547), p=0.0012 by t test.
- MSI-H CRC tumors (Lynch status unknown) (N=40) carry high mutations per case while Lynch syndrome tumors carry fewer mutations per case, which may be due to lower average age of Lynch syndrome patients.

Conclusions

- We used a combination of MSI fragment analysis and IHC to identify MSI phenotype in a large cohort of colorectal tumors.
- Using immunohistochemistry and NextGen sequencing, molecular features that are common to MSI-H CRC tumors and those that are specific to sporadic MSI-H tumors were identified.
- Significantly higher TS expression is a characteristic of both sporadic MSI-H and Lynch tumors, potentially explaining the observed reduced clinical benefit from 5-FU.
- Higher PD-1+ TIL, BRCA1/2 and CTNNB1 mutations suggest MSI-H as a more promising group for targeted immunotherapy, PARP and Wnt pathway inhibitors.
- Using NextGen sequencing, a significantly higher mutation load was observed in MSI-H tumors.
- Significantly higher mutation rates of PTEN, STK11 and FBXW7 observed in sporadic MSI-H tumors suggests that agents targeting PI3K/Akt/mTor pathway may be of particular interest in this subgroup of patients.

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