Comparative molecular analyses of BRAF-V600E mutant tumors: colorectal cancer (CRC) vs. melanoma

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Abstract (No. 3598)

Background: Little is known about the molecular characteristics of BRAF mutant (mt) CRC. It is unknown whether BRAF mt CRC have molecular and biological profile similar to BRAF mutant melanomas (Mel).

Methods: A total of 5139 tumor samples (CRC, 4007 and Mel, 1132) submitted to Caris Life Sciences for IHC (protein expression), ISH (gene amplification) and NGS sequencing between 2009 and 2015 were retrospectively studied. Chi-square tests determined differences.

Results: The rate of BRAF-V600E mutation in CRC was 7% (n = 270), and 30% in Mel (n = 334). Most frequently co-mutated genes in BRAF mt CRC were TP53 (56%), APC (26%), and PIK3CA (19%) and most frequently overexpressed proteins were TOP2A (90%), EGFR (77%), and cMET (57%). Most frequently co-mutated genes in BRAF mt Mel were CDKN2A (28%), ROS1 (19%); TP53(13%), and most frequently overexpressed proteins were PD1+TILs (75%), TS (71%), and TOP2A (68%). When compared to BRAF mt Mel, mt CRC tumors had a greater frequency of TP53 (56% vs. 13%), APC (26% vs. 3%), PIK3CA (19% vs. 1%), and SMAD4 (18% vs. 0%) mutations (all p-values < 0.01), whereas mutations in CDKN2A (28% vs. 19%) and ROS1 (19% vs. 12%) appeared higher in Mel (statistical significance not reached). In addition, BRAF mt CRC had a higher frequency of P-glycoprotein (PGP) (52% vs. 9%), cMET (57% vs. 13%), EGFR (77% vs. 6%), and HER2 (4% vs. 0%) overexpression (all p-values < 0.001), compared with Mel. However, PD1+TILs and ERCC1 were significantly higher in Mel (75% vs. 61%; p = 0.012; and 41% vs. 17%; p = 0.004). Co-occurring RAS mutations were rare, seen in 3 CRC and 2 Mel pts. MEK1 (1/31) and MEK2 (1/31) mutations were detected only in Mel. Mismatch repair deficiency and microsatellite instability (MSI) were seen in 34% of BRAF mt CRC. On examining PD1+TILs and PDL1 tumor expression in MSI-high (H) and MSI–stable (S) CRC, and comparing with Mel, PD1+TILs were found in 75% of Mel, 80% of MSI-H CRC, and 56% of MSI-S CRC (Mel or MSI-H CRC vs. MSI-S CRC; p < 0.01). PDL1 was positive in 10% of MSI-H and 15.8% of MSI-S CRC and 15.2% of Mel.

Conclusion: BRAF mt CRC may carry molecular and genetic alterations that are distinct from BRAF mt melanoma, suggesting different carcinogenic pathways and potential resistance mechanisms to therapy.

Background

- Oncogenic activation of BRAF occurs with highest frequency in thyroid (>30%), melanoma (>30%) and colorectal cancers (5-10%)
- The majority of BRAF mutations in thyroid, melanoma and colorectal cancers is the oncogenic, V600E. Additional variants at this residue, including V600K, K601E and V600R, result in activation of signaling.
- Targeting of BRAF with selective inhibitors have yielded response rates in the range of 48-52% in melanoma[1], but have not been as successful in colorectal cancers, with monotherapy response rates of 5%[2] and when combined with EGFR mabs or MEK inhibitors, only 12%[3].
- We investigated a biomarker database of molecularly-profiled CRC and melanoma for differences in biomarker patterns in BRAF-mutated tumors for further insight as to why response to BRAF inhibitors is so different between these two tumor types.

Methods

- 4,007 CRC tumors and 1,132 melanoma tumors were identified for having BRAF mutation status available. BRAF mutation status was determined by either the Cobas® 4800 V600 mutation test (Roche) or next-generation sequencing platforms (Illumina MiSeq and NextSeq).
- The following BRAF mutations were considered for inclusion into the BRAF V600-mutant patient subsets: V600E, V600E(2), V600K, V600R and K600_K601delinsE.
- Each submitted specimen was submitted for molecular profiling at a CLIA-certified laboratory, Caris Life Sciences, and included one more of the following assays: protein expression by immunohistochemistry (IHC), genomic copy number changes (in situ hybridization [ISH] or next-generation sequencing [NGS]) and mutational analysis (Illumina MiSeq or NextSeq NGS).
- Microsatellite instability 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). Standard protocol for determining unstable and stable status were used.
- Chi-square test (SPSS v.23, IBM; Armonk, NY) was utilized to test for significant differences between subgroups. A two-tailed *p* value ≤ 0.05 was considered statistically significant and Bonferroni correction was used to correct for multiple comparisons.
- All cutoffs, antibodies or probes used for analyses are available upon request.

Results

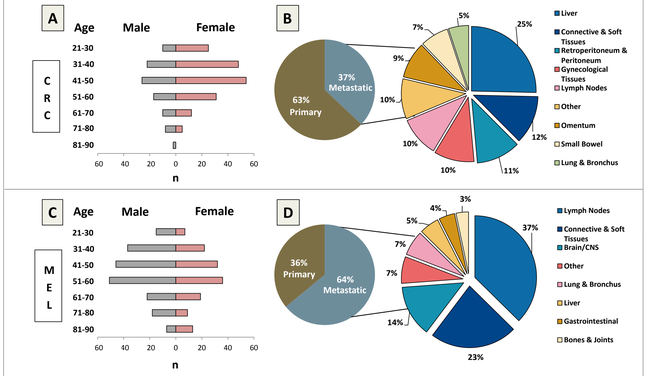


Figure 1. Distribution of age and gender (A,C) and specimen sites utilized for profiling (B,D) for V600-mutated CRC (top) and mel (bottom).

Results

Table 1. BRAF Mutation Frequencies in CRC and MEL

BRAF Mutation Status % (n)	CRC (4,007)	MEL (1,132)
V600	**6.7% (270)**	**29.5% (334)**
V600E	99.6% (269)	74.3% (248)
V600K	-	22.8% (76)
V600R	-	2.4% (8)
Other	0.4% (1)	0.6% (2)
Non-V600	**1.7% (70)**	**7% (79)**
BRAF wild-type	91.5% (3667)	63.5% (719)

Table 2. BRAF Status vs. Gender

Gender	CRC (270)	MEL (334)	p-value
Male	35% (95)	**58% (195)**	<0.001
Female	**65% (175)**	42% (139)	

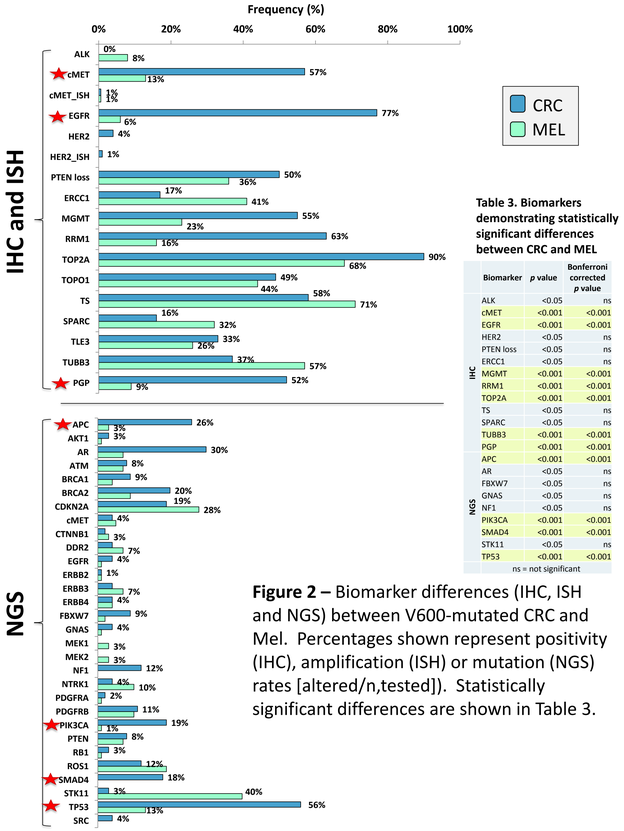


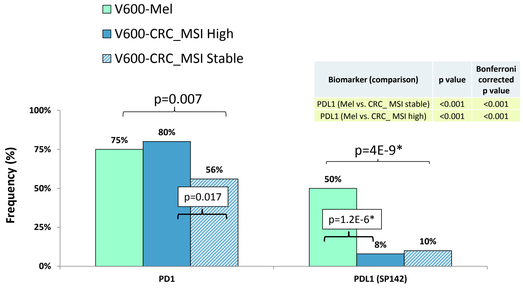
Table 3. Biomarkers demonstrating statistically significant differences between CRC and MEL

	Biomarker	*p* value	Bonferroni corrected *p* value
IHC	ALK	<0.05	ns
	cMET	<0.001	<0.001
	EGFR	<0.001	<0.001
	HER2	<0.05	ns
	PTEN loss	<0.05	ns
	ERCC1	<0.05	ns
	MGMT	<0.001	<0.001
	RRM1	<0.001	<0.001
	TOP2A	<0.001	<0.001
	TS	<0.05	ns
	SPARC	<0.05	ns
	TUBB3	<0.001	<0.001
	PGP	<0.001	<0.001
NGS	APC	<0.001	<0.001
	AR	<0.05	ns
	FBXW7	<0.05	ns
	GNAS	<0.05	ns
	NF1	<0.05	ns
	PIK3CA	<0.001	<0.001
	SMAD4	<0.001	<0.001
	STK11	<0.05	ns
	TP53	<0.001	<0.001

ns = not significant

Figure 2 – Biomarker differences (IHC, ISH and NGS) between V600-mutated CRC and Mel. Percentages shown represent positivity (IHC), amplification (ISH) or mutation (NGS) rates [altered/n,tested]). Statistically significant differences are shown in Table 3.

Results



Biomarker (comparison)	p value	Bonferroni corrected p value
PDL1 (Mel vs. CRC_ MSI stable)	<0.001	<0.001
PDL1 (Mel vs. CRC_ MSI high)	<0.001	<0.001

Figure 3 – PD1 and PDL1 in V600-mutated CRC and Mel. PD1 positivity is detected in tumor infiltrating lymphocytes (TILs) and PDL1 (SP142) expression is detected in tumor cells. Cutoffs: PD1 (≥ 1+), PDL1 (≥2+ and ≥5%).

Conclusions

- Several biomarkers tested by IHC exhibited differences in expression between V600-mutated CRC and Mel. After correction for multiple comparisons, EGFR, cMET, PGP, RRM1, MGMT, TOP2A and TUBB3 positivity rates were all increased in CRC, with the exception of TUBB3 which was increased in Mel.
- NGS markers displaying differences after correction for multiple comparisons included APC, PIK3CA, SMAD4 and TP53. The mutation rates for these genes occurred with higher frequency in CRC.
- PDL1 positivity rates in Mel was also significantly higher than both MSI-high and –stable CRC. Significance was maintained even after correction for multiple comparisons.
- The biological significance of these biomarkers and their role in differential responses to BRAF-inhibitors, whether having direct pharmacological effect on therapy, like P-glycoprotein, or indirectly, by creating a tumor phenotype more or less responsive to BRAF-inhibition, is currently under investigation.

References

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2. Kopetz, S. , L. Saltz, et al. (2015). "Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer ." J Clin Oncol 33(34):4032-8.
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