

Molecular profiling of melanoma brain metastases (MBM) compared to cutaneous melanoma (CM)

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Background

- Survival for metastatic melanoma has improved since 2011, due to improvements in systemic therapy, including immune checkpoint inhibitors and targeted agents. However, brain metastases (MBM) remain a significant cause of morbidity and mortality in patients with metastatic melanoma.
- Recently, combined checkpoint inhibition (CTLA-4 + PD-1), and combination targeted therapy (BRAF + MEK) were both shown to have efficacy in treating brain metastases, with efficacy similar to that of treating systemic disease.^{1,2} Yet there are no defined biomarkers to guide treatment for patients with resistant MBM.
- The biological underpinnings of melanoma brain metastases, as compared to other melanoma tumors (primary, non-CNS metastases) remains unclear. Increasing evidence suggests a distinct evolution, with unique molecular features.³ Activation of the MAPK pathway, as well as the PI3K-AKT pathway, have been implicated in the pathogenesis of MBM.⁴
- Herein, we seek to understand the interplay between PD-L1, TMB, BRAF, and other oncogenic pathways among melanoma brain metastases (MBM), as compared against cutaneous melanoma (CM) and other non-CNS melanoma metastases (MOM).

Methods

- Tumors submitted to Caris Life Sciences (Phoenix, AZ) for routine molecular profiling between January 2015 and January 2018 were reviewed from a de-identified database. We analyzed a total of 132 MBM, 745 CM and 1190 MOM.
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq (592-genes)/MiSeq platform (45-gene) (Illumina, Inc., San Diego, CA). All variants were detected with greater than 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage greater than 500 and an analytic sensitivity of 5%.
- Microsatellite instability (MSI) was examined by counting number of microsatellite loci that were altered by somatic insertion or deletion counted for each sample. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of > 95% and specificity of > 99%.
- Tumor mutational burden (TMB) was estimated from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations. TMB was determined as high or low using a threshold of ≥ 17 mutations/Mb.
- IHC was performed on FFPE sections of glass slides. PD-L1 testing was performed using the SP142 (Ventana, Tucson, AZ) anti-PD-L1 clone as measured on tumor cells. PD-L1 positivity was evaluated using a threshold of 1+ staining intensity on $\geq 1\%$ of tumor cells.
- Comparison of molecular profiles, including cancer-related genes and recurrently altered pathways, between tumor sites, and by genomic subgroup (BRAF, NRAS, KIT, NF1), Chi-square, t-tests, and Wilcoxon test were performed for comparative analyses using R (version 3.5.0).

Results

	CM	MBM	MOM	p-value
Age at collection date				0.0001
Median	67	62	64	
Range	0-96	25-83	16-95	
Gender				0.2003
Female	262 (35%)	36 (27%)	413 (34%)	
Male	483 (64%)	96 (72%)	777 (65%)	

Table 1. Patient demographics.

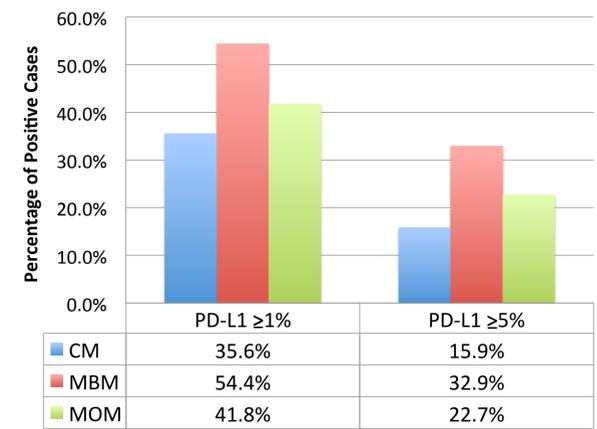


Figure 1. Frequency of PD-L1 expression. MBM showed higher PD-L1 expression, using a $\geq 1\%$ ($p=0.002$) and $\geq 5\%$ cut-off ($p=0.0006$)

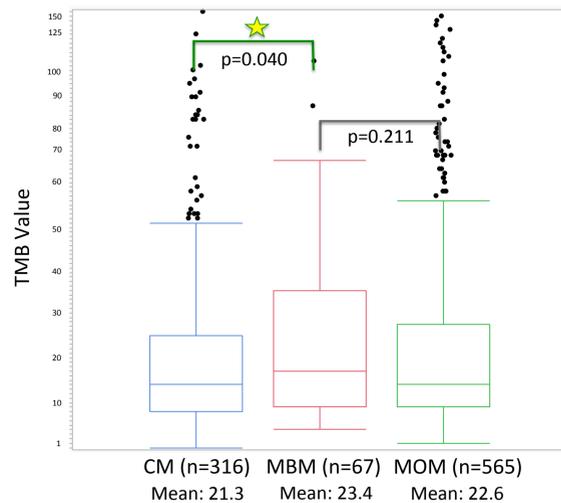


Figure 2. Distribution of TMB across melanomas. MBM had statistically higher TMB than CM, but not MOM, and was more frequently TMB-H (53.3% vs 38%, chi-sq $p=0.024$).

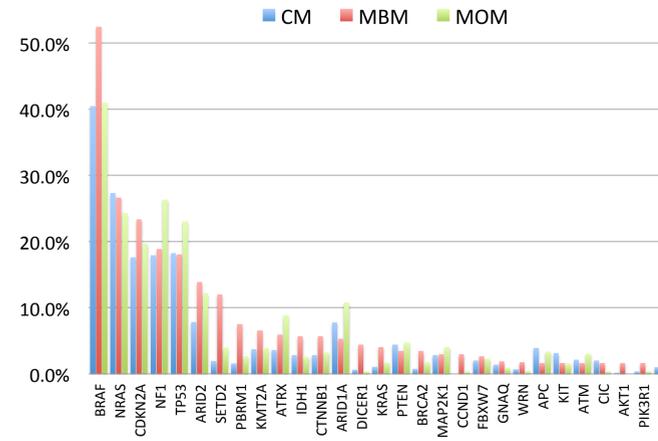


Figure 3. Comparison of top 30 most frequently altered genes across CM, MBM and MOM. Frequency of gene mutations or amplifications (amplifications are denoted with a *) detected in a cohort of melanomas from diverse specimen sites.

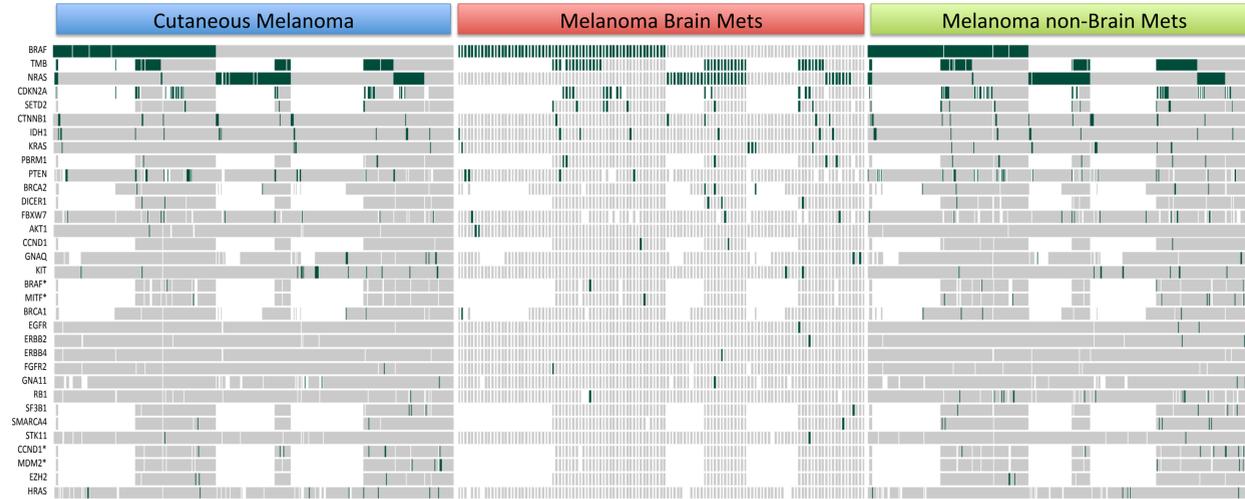


Figure 4. Differentially altered genes in melanomas from diverse specimen sites. Frequency of differentially altered genes (amplifications are denoted with a *) detected in a cohort of melanomas. Green bars indicate the presence a pathogenic mutation or gene amplification, gray bars indicate no oncogenic alteration detected, and white bars indicate no testing was performed. MBM showed higher rates of GAs among: *SETD2* (11.9% v 1.9%, $p=0.0008$), *BRAF* (52.4% v 35.6%, $p=0.017$), *PBRM1* (7.5% v 1.6%, $p=0.018$), *KRAS* (4% v 1%, $p=0.026$), *CCND1* (2.9% v 0%, $p=0.03$), and *DICER1* (4.4% v 0.6%, $p=0.04$), compared to CM.

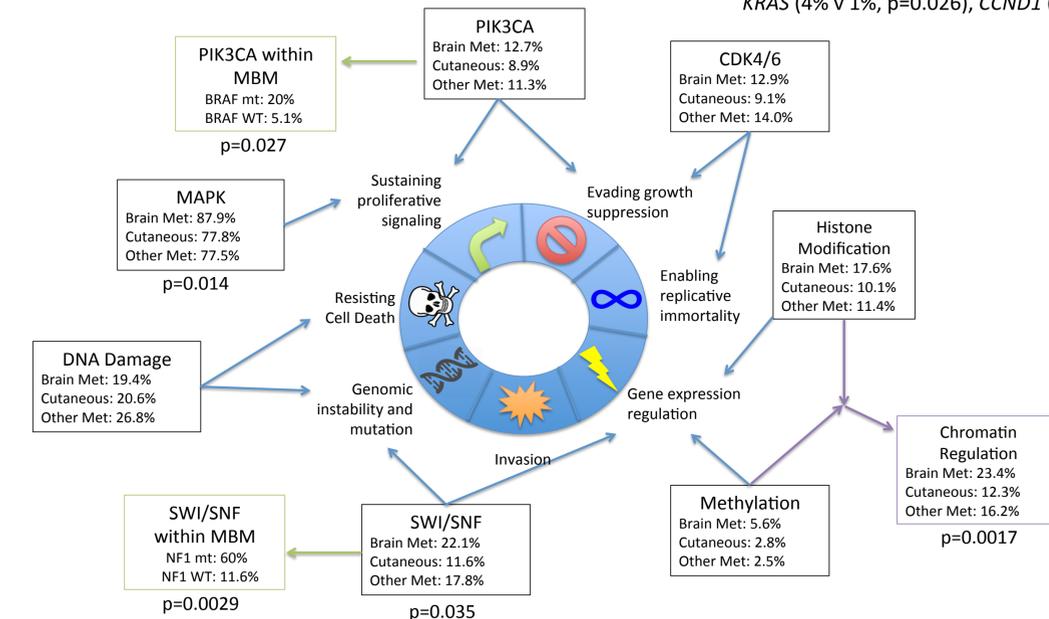


Figure 5. Grouped pathway analysis. Genes were grouped by the signaling pathway in which they belong. Tumors were labeled as altered at the pathway level if they harbored an alteration within one or more genes within each pathway. Frequency of pathway-level alteration was compared between tumor cohorts. Alterations of MAPK (88% v 78%, $p=0.015$), SWI/SNF (22% v 12%, $p=0.036$), and chromatin regulation (23% vs 12%, $p=0.0017$) pathways were more frequent in MBM, than CM. PIK3CA pathway alterations were more frequent among BRAF mut MBM, compared to BRAF wt (20% v 5%, $p=0.027$), while SWI/SNF alterations were more frequent among NF1 mut MBM (60% v 12%, $p=0.0029$), compared to NF1 wt.

Conclusions

- Melanoma brain metastases (MBM) demonstrate a unique molecular profile, when compared to primary cutaneous melanoma (CM) and other non-CNS melanoma metastases (MOM).
- MBM were associated with higher rates of BRAF mutations, as well as higher TMB and higher PD-L1 expression.
- Genetic alterations among genes associated with epigenetic modification were frequently seen among MBM. We noted significant alterations among *PBRM1* and *SETD2*, which have not been previously identified in the context of MBM.
- Pathway analyses revealed higher rates of genetic alterations in the MAPK pathway, as well as SWI/SNF and chromatin remodeling pathways, among MBM.
- Our data suggests that epigenetic modification may play an important role in the biology of melanoma brain metastases, warranting further investigation.
- Ongoing studies will further analyze differences among other sites of melanoma metastases.

References

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