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 mortality and morbidity 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. Yet, there are no definite 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 genetic and epigenetic features. Activation of the MAPK pathway, as well as the PI3K-AKT pathway, have been implicated in the pathogenesis of MBM.

- Herein, we seek to understand the genetic landscape of recurrent BRAF-mutant MBM, and how it differs from primary cutaneous malignant melanoma (CM) and other non-CNS melanoma metastases (MOM).

Methods

- Tumors submitted to Caric Life Sciences (Phoenix, AZ) for routine molecular profiling between January 2015 and January 2018 were reviewed for a de-identified database. We analyzed a total of 132 MBM. 74 CM and 110 MOM.

- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq500 (N500) platform (Illumina, Inc., San Diego, CA). All variants were detected with greater than 99% confidence based on allele frequency and amplification coverage, with an average sequencing depth of 10-fold per base and a 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 92 genes [14 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 27 mutations/MB.

- IHC was performed on FFPE sections of glass slides. PD-1+ testing was performed using the 2842 (Ventana, Tucson, AZ), anti-PD-L1 clone 1.8.1.32 as a surrogate for PD-L1 positivity. PD-L1 positivity was evaluated using a threshold of 1+ staining intensity on ≥30% of tumor cells.

- Comparison of molecular profiles, including cancer 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 analysis and intrapair analysis (version 3.5.0).

Results

- Frequency of PD-L1 expression. CM showed higher PD-L1 expression, using a cut-off of >20% with a cut-off of 1%.

- TMB was statistically higher in MBM than in CM, but not MOM and was more frequently high TMB in (53.5% vs 38%, chi-sq p= 0.024).

- Frequency of gene mutations or amplifications (amplifications were detected with a *) in a cohort of melanoma from diverse specimen sites.

- Microsatellite instability (MSI) was examined by MSI status of tumors. MSI-H was present in a higher percentage of MBM compared to CO.

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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 MSI with a higher frequency of BRAF and NRAS mutations.

- Genetic alterations among genes associated with epigenetic modification were frequently seen among MBM. We noted significant alterations among PBRM1 and CDKN2A, 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.

- Future studies will further analyze differences among other sites of melanoma metastases.

References


