Whole Transcriptome Sequencing Reveals Oncogenic Fusions in Melanoma

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Introduction

- Somatic genomic alterations occur frequently in melanoma
- *BRAF* mutations are the most clinically relevant as they predict for response to targeted therapies
- Oncogenic gene fusions are frequently identified in different cancers with an unknown incidence in melanoma
- Targeted therapies are approved for specific gene fusions in other tumor types and are now standard of care
- The aim of this retrospective study was to determine the prevalence of oncogenic fusions in metastatic or locally advanced melanoma
- Gene expression analysis across a broad group of melanomas with and without fusions was performed in order to better elucidate the functional consequences of gene fusions in this aggressive malignancy

Methods

- Retrospective analysis of FFPE patient tumors that were profiled as part of routine clinical testing (Caris Life Sciences, Phoenix, AZ)
- Samples were profiled by next-generation sequencing of DNA/RNA (592-gene panel/whole transcriptome [WTS]) and immunohistochemistry
- In addition to detection of fusions by WTS, samples were exampled for co-alterations, including tumor mutational burden (TMB), deficient mismatch repair/high microsatellite instability (dMMR/MSI-High), and PD-L1 protein expression.

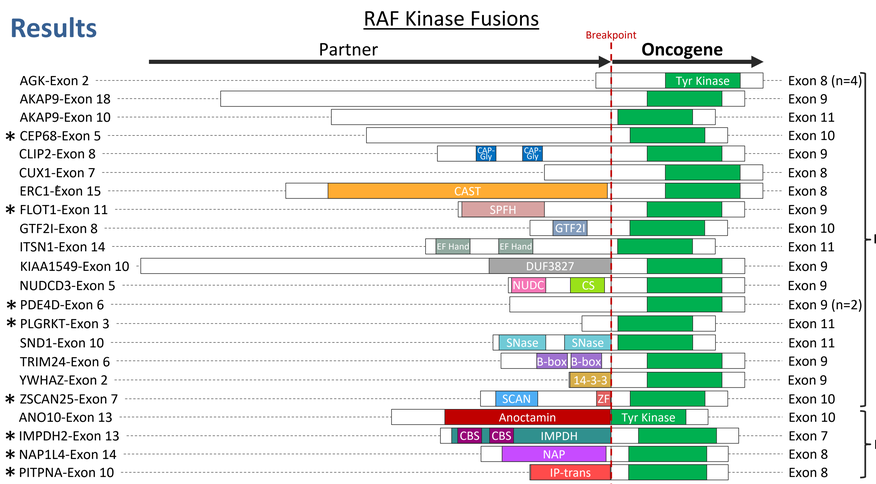
Results

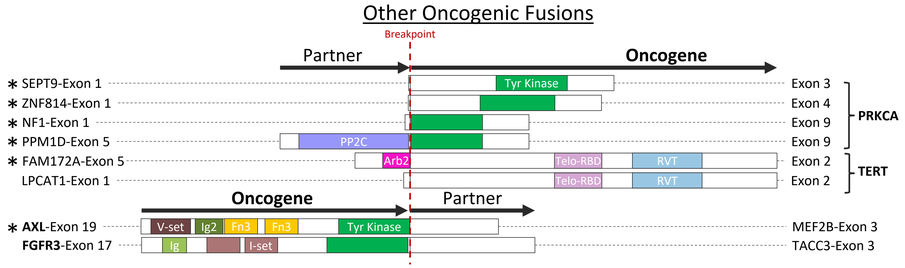
- 1,255 melanoma specimens were screened for fusions (Table)
- We identified 33 (2.6%) tumors with in-frame oncogenic fusions (Figures 1a and 1b)
  - 25 Raf kinase fusion: 21 *BRAF* fusions and 4 *RAF1* fusions
- 796 (63.4%) cases with *RAS/RAF* pathogenic or likely pathogenic mutations
  - 373 (30.0%) *BRAF* p.V600X mutations
- Tumors harboring *PRKCA* and *TERT* fusions were each detected with at least one MAPK pathway co-alteration (*NRAS, NF1*, or *BRAF* p.V600E mutation)

Patient/Tumor Characteristics	
Total, N cases	1,255
Median Age, years (SD) - Age Range, years	67 (13.5) 3-90+
Female/Male, N cases - (% Female/% Male)	478/777 (38.1%/61.9%)
Metastatic/Primary, N cases - (% Metastatic/% Primary) - [N unclear]	780/456 (63.1%/36.9%) [19]

Table. Patient/Tumor Characteristics

Results





↑Figure 1b. Other Oncogenic Fusions
Identified 8 non-RAF Kinase fusion involving PRKCA (4), TERT (2), AXL (1), and FGFR3 (1), including 6 novel (*) fusions.

←Figure 1a. RAS Kinase Fusions
We identified 25 patients with fusions involving *BRAF* (21) or *RAF1* (4) genes, including 9 novel (*) fusions.

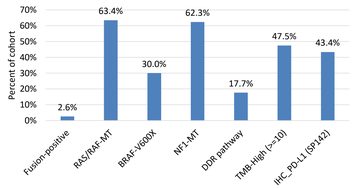


Figure 2. Fusion prevalence compared to other biomarkers in melanoma. Oncogenic fusions were rare (2.6%, n=33 samples) compared to other RAS/RAF pathway alterations (63.4%), including BRAF-V600X (30.0%) and NF1 mutations (62.3%). TMB-High and/or PD-L1 expression were observed in nearly half (47.5% and 43.4%, respectively) of all melanoma samples examined.

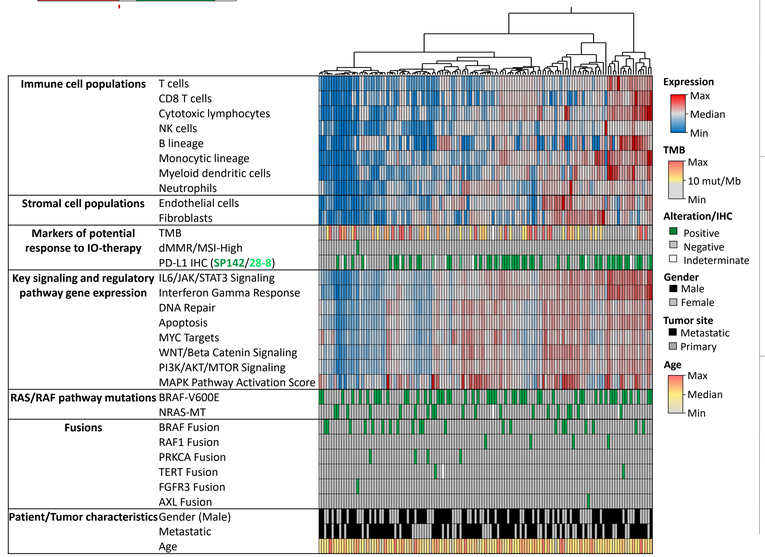


Figure 3. Expression analysis.
The heatmap shows *BRAF* fusions have a trend toward low immune cell abundance and increased stromal population. Hierarchical clustering based on immune and stromal cell population abundance.

Study Highlights

- 33 patients with an in-frame oncogenic fusions identified
  - Including 15 novel fusions
  - 10 (32.3%) exhibited PD-L1 expression by IHC (SP142 or 28-8)
  - 11 (33.3%) were TMB-High
- Fusion transcripts with unknown pathogenicity were detected in 668 (53.2%) of melanoma samples
  - 2,404 total unclassified fusion isoforms detected
  - 10 recurrent fusions (≥ 3 occurrences)
- RNA expression analysis of Wnt/β-catenin, PI3K/AKT/MTOR, DNA repair, INFG, and JAK/STAT pathways showed a high degree of variability among fusion-positive tumors
- DNA damage repair pathway mutation frequency similar in patients with fusion-positive and fusion-negative tumors

Conclusions

- Oncogenic gene fusions are rare in melanoma
- Potentially actionable fusions, in addition to co-alterations in fusion-positive cases, can be identified through comprehensive tumor molecular profiling
- Clinical trials or targeted treatments would be an options for patients with fusion-positive advanced melanoma

Selected References

Birkeland, E., Zhang, S., Poduval, D., Geisler, J., Nakken, S., Vodak, D., ... & Lønning, P. E. (2018). Patterns of genomic evolution in advanced melanoma. Nature communications, 9(1), 2665.
Joseph, E. W., Pratilas, C. A., Poulikakos, P. I., Tadi, M., Wang, W., Taylor, B. S., ... & Rosen, N. (2010). The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. Proceedings of the National Academy of Sciences, 107(33), 14903-14908.
Kumar-Sinha, C., Kalyana-Sundaram, S., & Chinnaiyan, A. M. (2015). Landscape of gene fusions in epithelial cancers: seq and ye shall find. Genome medicine, 7(1), 129.
Quan, V. L., Panah, E., Zhang, B., Shi, K., Mohan, L. S., & Gerami, P. (2019). The role of gene fusions in melanocytic neoplasms. Journal of cutaneous pathology.

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