Molecular and immunologic characterization of HRAS mutations in a cohort of 6,329 patients with cutaneous melanoma

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BACKGROUND

Activation in *RAS* pathway has been associated with cancer development. The oncogenes of *RAS* family (NRAS, KRAS and HRAS) are frequently mutated across various cancer types, where *NRAS* mutations are present in 15-20% of melanomas. *NRAS*-mutant melanomas (*NRASm*) have been extensively characterized. However, molecular and clinical implications of *HRAS* mutations (*HRASm*) in melanoma are less well understood.

METHODS

- A total of 6329 melanoma samples were subjected to next generation sequencing of DNA (592 Gene Panel, NextSeq; whole exome sequencing, NovaSEQ), RNA (NovaSeq, whole transcriptome sequencing, WTS) and IHC.
- MPAS scores to evaluate MAPK pathway activation (Wagle et al, Precision Oncology), IFN scores (Cristescu et al., Science), QuantiSeq, neoantigen load (high, intermediate, low binding affinity: HBA, IBA and LBA) and GSEA were calculated from mRNA expression data.
- Wilcoxon, Fisher's exact were used to determined statistical significance (p value without and q value with multi comparison correction; FDR for GSEA). The reference cohort was the entire melanoma cohort (MC).

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RESULTS

Genomic Landscape of HRAS mutation

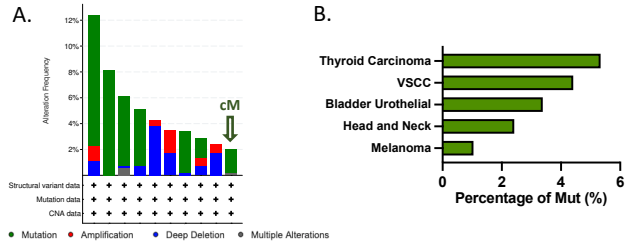

Figure 1: Prevalence of *HRASm* in different tumor types in TCGA (A, cM: cutaneous melanoma) and Caris Database (B, VSCC: vulvar squamous cell carcinoma)

Table 1-2: Clinicopathological features associated with *HRASm* and prevalence

	HRASm	General Cohort	p	q
Count(N)	50	4401		
Average Age (range)	69.5 (39 - >89)	65.1 (0 - >89)	0.03	0.13
Male	72.0% (36/50)	62.8% (2766/4402)	0.18	0.37
Female	28.0% (14/50)	37.2% (1636/4402)		

Cohorts	Melanoma (N = 4401)			
	Mutation (N= 50, 1.13%)			
	Q61	G12	G13	other
Point mutation N	16	11	19	4

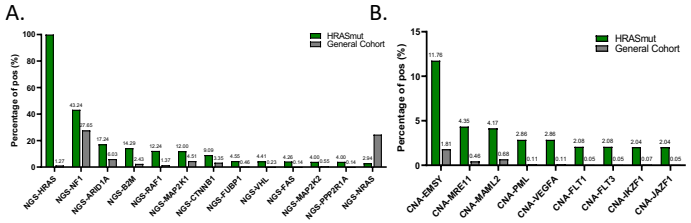

Figure 2. Significantly different genomic co-alterations (A, mutation; B, copy number amplification (CNA), $p < 0.05$) in *HRASm* with respect to GC.

- HRAS mutational status was not associated with either gender or age
- HRAS mutants harbored significantly more *NF1, ARID1A, B2M, RAF1, CTNNB1* mutations and were almost mutually exclusive with NRAS mutation; they are also associated with amplifications of EMSY, MRE11, MAML2; G13 mutants carried the most alteration of NF1 mutation.

CONCLUSIONS

HRAS-mutated melanomas in this cohort had a different molecular and immunologic landscape compared to *HRAS*-wildtype and *NRAS*-mutated tumors.

HRAS-mutated melanomas showed higher MAPK activation, down-regulation of angiogenesis pathway, and more immunogenic features suggesting a potential effect of the oncogene (*HRAS*) mutation on the tumor microenvironment and higher susceptibility of *HRASm* to immunotherapy.

HRAS vs NRAS in melanoma - Genomic

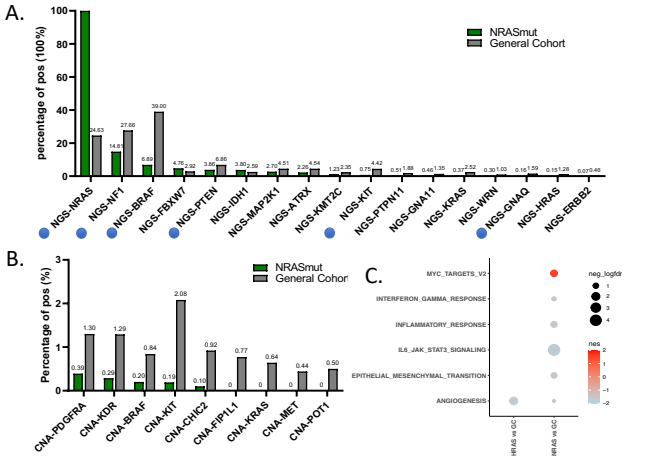

Figure 3: Significantly different genomic co-alterations. A, mutation; B, copy number amplification (CNA)) in *NRASm* with respect to GC and C, differential pathway regulations of *HRASm* and *NRASm* compared to GC.

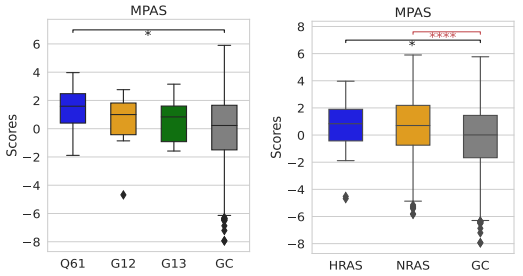

Figure 4: MPAS scores in HRAS point mutation (left) and *HRASm, NRASm* and GC

- NRAS mutants had different genomic landscape from HRAS mutants. They were also associated with significantly less *NF1, BRAF, PTEN, KIT* and *GNAQ* mutations (both p value and q value significant);
- *HRASm* had higher MPAS scores and HRAS Q61 displayed the highest MPAS score with respect to GC.
- *HRASm* had higher neoantigen load with high/intermediate binding affinity to MHC proteins and displayed a trend to higher infiltrates of CD8+ T cells and CD4+ T cells.
- Majority of tumors with both *HRASm* and NF1 mutations are TMB-high.

HRAS vs NRAS in melanoma - Immunologic

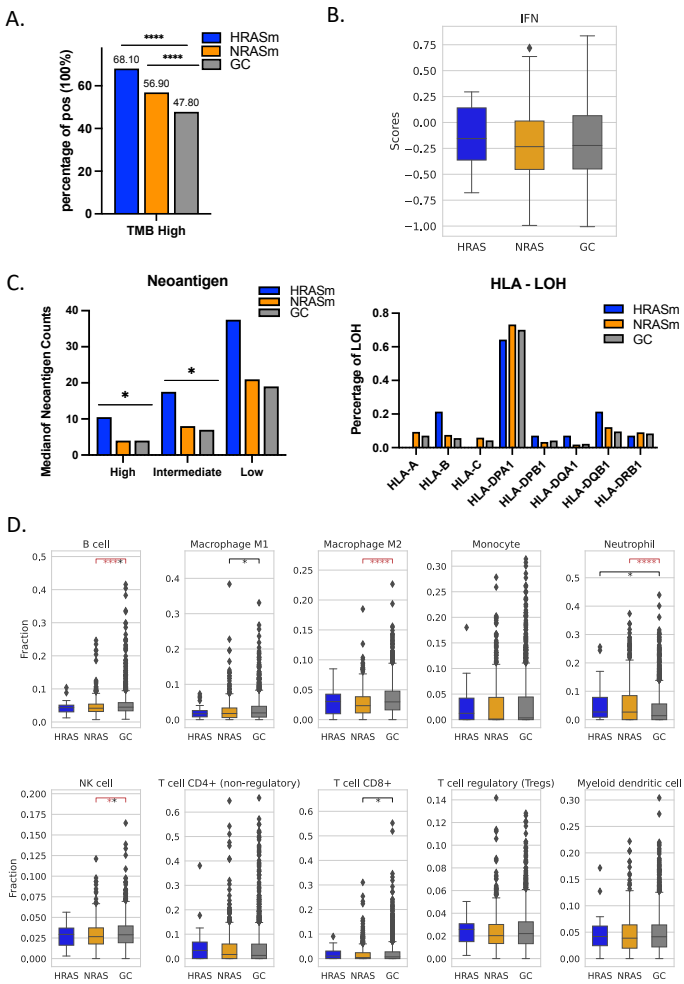

Figure 5. Immunological landscape characterization in *HRASm, NRASm* vs. GC (A, TMB; B, IFN score; C, Neoantigen load; D, HLA loss of heterozygosity).