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Comparative analysis of the *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX* driver mutations in melanoma and across the cancer spectrum

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Abstract

Uveal melanoma is characterized by recurrent mutations in *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX*, as well as a low total mutational burden. The frequency and clinical significance of these mutations in non-uveal melanoma and other cancers is not well described. We identified that *GNAQ*/*GNA11* mutations occur in 0.5–1% of non-uveal melanomas and are essentially melanoma-specific. Further, these mutations are associated with a lack of other typical melanoma mutations (*BRAF*, *NRAS*, *KIT*, *NF1*), a low mutational burden, and, in a small subset, lack of response to immunotherapy. We suggest that *GNAQ*/*GNA11* mutations characterize an uncommon but distinct subtype of non-uveal melanomas.

Keywords

GNAQ; GNA11; SF3B1; EIF1AX; uveal; melanoma; mutation; cutaneous

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Dear Editor

Uveal melanoma (UM) is an uncommon subtype of melanoma with distinct clinical and molecular features. While novel immune and targeted therapies have revolutionized treatment for non-uveal melanoma (NUM), these agents have suboptimal efficacy in UM (Carvajal et al., 2014; Luke et al., 2013; Maio et al., 2013). Further, recurrent "driver" mutations observed in NUM, such as those in *BRAF*, *NRAS*, and *KIT*, are essentially absent in UM. Instead, recurrent mutations in *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX* predominate in UMs (Harbour et al., 2013; Van Raamsdonk et al., 2009; Van Raamsdonk et al., 2010). *GNAQ*/*GNA11* codon 209 mutations induce mitogen-activated protein kinase (MAPK) signaling and occur in ~80% of UMs (and in rare melanocytic neoplasms: blue nevi, central nervous system melanocytomas). The frequency, genetic profile (including co-occurring mutations), and clinical implications of these stereotypical UM mutations in NUM and other cancers have not been thoroughly characterized.

To investigate, we profiled large next generation sequencing (NGS) databases including The Cancer Genome Atlas (TCGA) to facilitate systematic assessment of these alterations and co-occurring mutations across the spectrum of human cancers (Cancer Genome Atlas Network, 2015). We then reviewed the sequencing data from >2000 patients from four large melanoma centers (Vanderbilt, MD Anderson, Memorial Sloan Kettering, Moffitt Cancer Centers), to characterize the clinical and pathologic features of melanomas harboring these mutations.

Across the cancer spectrum in the TCGA (17 malignancies, 4972 individual samples) and two other publically-available melanoma databases, we did not identify any *GNAQ*/*GNA11*^{Q209} mutations outside of cutaneous melanomas (non-cutaneous melanomas were excluded from the TCGA). Sporadic cases of non-Q209 *GNAQ*/*GNA11* mutations were identified in various malignancies (<1% incidence); all were distinct and non-recurrent (Table S1). Next, we assessed the frequency of *SF3B1* missense mutations in previously determined "hotspots" (codons 622, 625, 626, 662, 666, 700, 742). These were identified in breast carcinoma (1.1% of samples) and other cancers (Table S1). Similar to UM, *SF3B1*$^{R625C/H}$ mutations were enriched in NUM compared to other "hotspot" mutations (6/8 samples, 75%). By contrast *SF3B1*^{K700E} mutations predominated in breast carcinoma (8/11 samples, 73%), similar to myelodysplastic syndrome and chronic lymphocytic leukemia. *EIF1AX* alterations occurred infrequently in low-grade gliomas (1.4%), uterus endometrial carcinoma (1.25%), thyroid carcinoma (1%), and lung adenocarcinoma (0.4%) (Table S1). *SF3B1* and *EIF1AX* mutations did not co-occur in any sample.

We then assessed the frequency of *GNAQ*, *GNA11*, *SF3B1*, and *EIF1AX* mutations in NUMs using three large publically-available melanoma sequencing databases (Cancer Genome Atlas Network, 2015; Hodis et al., 2012; Krauthammer et al., 2012). We identified two *GNAQ*^{Q209P} and two *GNA11*^{Q209} mutant samples, comprising 0.83% of the total NUMs (4 of 483). *SF3B1* "hotspot" mutations were present in 8 NUMs (1.66%), including three with concurrent *GNAQ/GNA11*^{Q209} mutations. Three NUMs harbored *EIF1AX* mutations (0.62%) and overlapped with a *GNA11*^{Q209} mutation in one case. Notably, activating *GNAQ*/*GNA11* mutations did not co-occur with other common melanoma

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"driver" mutations affecting MAPK signaling (e.g., *BRAF*, *NRAS, KIT*) in NUM, supporting the notion that activated *GNAQ*/*GNA11* is sufficient to activate the MAPK pathway (Figure 1). By contrast, the 8 samples with *SF3B1* or *EIF1AX* mutations without *GNAQ*/*GNA11* mutations, frequently overlapped with $BRAF^{V600}$ mutations (n=4) or $NRAS^{Q61}$ mutations (n=3).

A high burden of somatic nonsynonymous exomic mutations (and thus a greater repertoire of neo-antigens) has been correlated with greater response to immune checkpoint inhibitors (Snyder et al., 2014; Van Allen et al., 2015); UM has an extremely low mutation burden (Krauthammer et al., 2012). In the TCGA, *GNAQ*/*GNA11*-mutated NUMs had a substantially lower mutational burden compared to those with *SF3B1*/*EIF1AX* mutations (without concurrent *GNAQ*/*GNA11* mutations) and all other NUMs (median mutations 35 vs. 251 vs. 287; p=0.02) (Figure S1A). Furthermore, only 1 of 3 *GNAQ*/*GNA11*-mutated tumors with evaluable UV-signatures possessed this signature, compared with 5 of 5 *SF3B1*/*EIF1AX*-mutated tumors, and 236 of 276 (85%) of others. Among clinical samples, targeted NGS (sequencing 236 genes) for 3 *GNAQ*/*GNA11*-mutated melanomas was performed; these samples had fewer mutations compared to 48 other unselected melanoma samples (median 8 vs. 17 mutations; p=0.03; Figure S1B). Notably, 4 tumors and matched germline from MSKCC were profiled by a different NGS platform assessing 411 genes (MSK-IMPACT); these had 2, 3, 4, and 49 mutations identified, respectively. To further characterize immune characteristics, we interrogated the melanoma TCGA for expression of genes corresponding to activated T-cell infiltrates (*CD8A*, *PD-L1* [*CD274*]) or IFN-γ signatures (*CCL4*, *CXCL9*). We found that all *GNAQ*/*GNA11*-mutated samples had minimal expression of these immune-related genes (Figure S1C–F), contrasting with other melanomas.

We then reviewed clinical sequencing data from 1950 melanoma samples from MDACC (n=664) and VICC (n=1286) and identified 12 NUM samples with *GNAQ/GNA11*^{Q209} alterations (0.62%). We also obtained all identified cases (n=5) from two other centers (Memorial Sloan Kettering and Moffitt Cancer Centers); clinical characteristics are in Table 1. These samples included cutaneous, mucosal, and unknown primaries, and included skin of intermittent and chronic sun exposure. These unknown primaries appeared unlikely to represent occult uveal melanoma; four patients had normal eye exams, and only two patients had liver metastases, by far the most common metastatic site for UM.

Among these 17 tumors with *GNAQ*/*GNA11*^{Q209} mutations, concurrent *BRAF*, *NRAS*, *KIT*, *NF1* or other MAPK-activating alterations were not identified, with one exception (vulvar melanoma with $GNA11^{Q209H}$, KIT^{V559D}, and $BRAF^{G469V}$ mutations). We then assessed treatment outcomes; of 11 patients with *GNAQ*/*GNA11* mutations who received immunotherapy, only one responded to treatment. These included 6 patients who received anti-PD-1 agents, and others treated with ipilimumab, high-dose interleukin-2, and/or autologous T cell infusions (Table 1). The single patient with an immunotherapy response had the only highly-mutated tumor in this group (49 mutations on targeted NGS as mentioned above).

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In conclusion, we identified that *GNAQ*/*GNA11*^{Q209} mutations characterized a distinct, albeit uncommon subtype of NUM (0.5–1%). These mutations were essentially melanoma-specific, occurred in all subtypes of this disease (including cutaneous, mucosal, uveal, and unknown primary), and were mutually exclusive with other common melanoma mutations. Further, in small number of samples, they were generally associated with extremely low mutational burden and lack of T cell markers. Thus, we speculate that this subtype could possibly have a poor response to immunotherapies but may be amenable to MAPK-directed targeted therapy strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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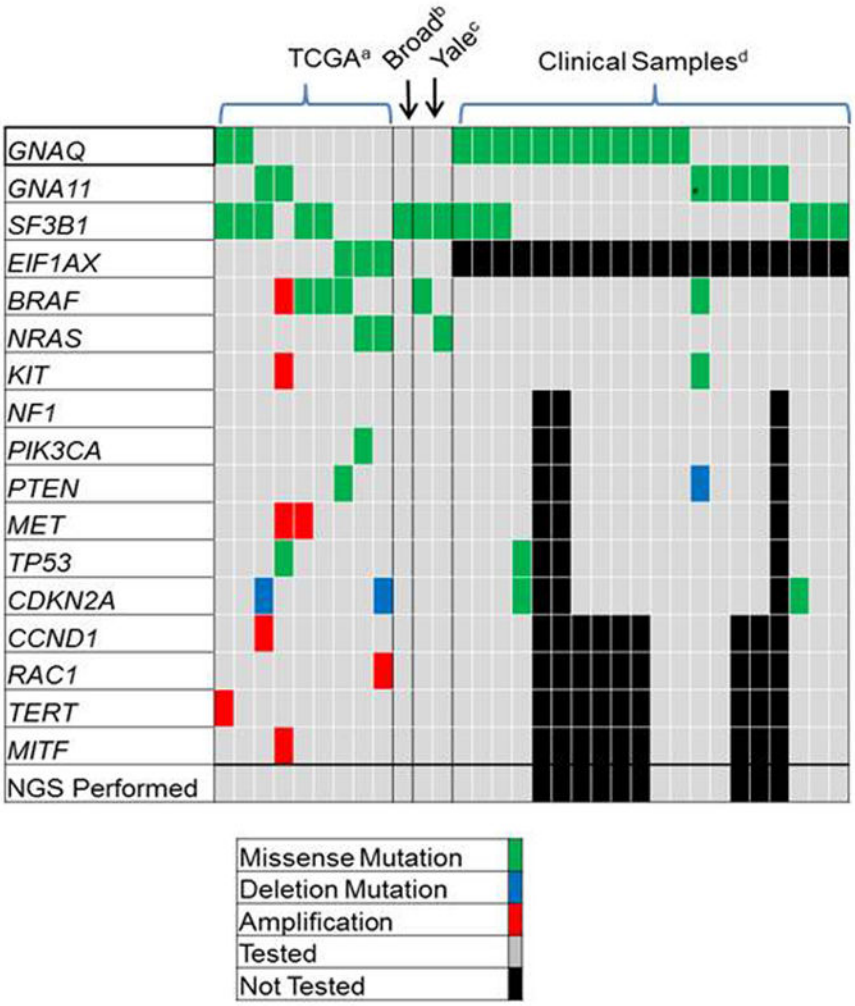

Figure 1.

Co-occurring genetic alterations in non-uveal melanomas harboring mutations in GNAQ, GNA11, SF3B1, and EIF1AX. Note: No mutations were identified in *NF1*, *KRAS*, *MAP2K1*, *AKT1*, *CTNNB1*, or *CDK4* [A]TCGA: The Cancer Genome Atlas, whole exome sequencing, 278 melanomas. [B]Broad: whole exome sequencing, 121 melanomas (mutations only) performed at the Broad Institute. [C]Yale: Whole exome sequencing, 91 melanomas (mutations only) performed at Yale. [D]Clinical Samples: Next generation sequencing or hotspot mutation testing performed at VICC or MDACC. **NRAS*^{A83V} mutation identified. #Co-occurring mutations in *KIT*^{V559D}, *BRAF*^{G469V}

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Table 1

Mutations in GNAQ, GNA11, and SF3B1 in clinical samples and corresponding clinical characteristics

Center	Testing	Mutation	Other mutations	Primary Site	Metastases	Age	Sex	Notes
VICC	PCR	*GNAQ*^{Q209P}	None	Neck	Liver, lung, bone	57	M	No response to IL-2 or ipi
VICC	PCR	*GNAQ*^{Q209P}	None	Unknown	Cerebellum	58	F	Rapid CNS progression
VICC	NGS	*GNAQ*^{Q209P}	*SF3B1*^{R625H}	Unknown	Retroperitoneal lymph nodes, lung, liver	37	F	No response to IL-2, ipi, or pembro
MDACC	PCR	*GNAQ*^{Q209P}	None	Anus	Liver	48	M	No response to ipi and temozolomide
MDACC	PCR	*GNAQ*^{Q209P}	None	Scalp	Cervical lymph nodes	70	F	No systemic therapy
MDACC	PCR	*GNAQ*^{Q209L}	None	Unknown	Pleura, lungs	68	M	Unknown systemic therapy
MDACC	PCR	*GNAQ*^{Q209L}	None	Thigh	Brain	37	F	Unknown systemic therapy
MSKCC	NGS	*GNAQ*^{Q209L}	*BAP1*$^{E31\_del}$	Scalp	Liver	27	M	No response to ipi or pembro
MSKCC	NGS	*GNAQ*^{Q209L}	*SF3B1*^{R625H}	Unknown	Brain	65	M	No response to ipi or pembro
MSKCC	NGS	*GNAQ*^{Q209L}	*SF3B1*^{R625C}	Unknown	Retroperitoneal mass	29	F	Stage IV oligometastatic resected
MCC	NGS	*GNAQ*^{Q209L}	None	Rectum	perirectal nodal mets	73	F	Ipilimumab with brief stable disease
MSKCC	NGS	*GNAQ*^{Q209H}	*CDKN2A*^{E88K}, *TP53*^{G127F}	Shoulder	Gastric; Lung; Adrenal	76	M	Excellent PR x 2 years to ipi
VICC	NGS	*GNA11*^{Q209L}	None	Unknown	Cervical lymph nodes	35	M	No response to nivo
VICC	PCR	*GNA11*^{Q209L}	None	Scalp	Skin	57	M	Isolated skin metastasis resected
VICC	NGS	*GNA11*^{Q209H}	*KIT*^{V559D}, *BRAF*^{G469V}, *PTEN* loss	Vulva	Brain, lungs, liver, bone	48	F	Partial response to sunitinib, no response to ipi or biochemotherapy
MDACC	PCR	*GNA11*^{Q209L}	None	Scalp	Liver	50	F	No response to nivo + ipi, adoptive T cell therapy, IL-2
MDACC	PCR	*GNA11*^{Q209L}	None	Unknown	Axillary lymph nodes, liver	70	M	No response to pembro or ipi
VICC	NGS	*SF3B1*^{R625H}	None	Vulvar	Lungs, liver, bones	60	F	No response to ipi
VICC	NGS	*SF3B1*^{K666N}	*ATM*^{S2408L}, *CDKN2A*^{G89S}, *CDKN2A*^{P81L}, *RAF1*^{S257L}	Forearm	Lungs	71	M	Durable partial response to ipi
MCC	NGS	*SF3B1*^{K666N}	None	Leg	in transit skin, colon	84	F	Ipi with 2 years stable disease, then no response to pembro
Blue nevi or primary CNS melanomas/melanocytomas								
MDACC	PCR	*GNAQ*^{Q209L}	None	Buttock	None	34	M	Blue nevus evolved into melanoma
MDACC	PCR	*GNAQ*^{Q209L}	None	Brain	None	62	M	Frontal lesion, craniotomy, no relapse

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Center	Testing	Mutation	Other mutations	Primary Site	Metastases	Age	Sex	Notes
MDACC	PCR	*GNAQ*^{Q209P}	None	Brain	None	40	M	Cerebellar/dural lesion, craniotomy, intrathecal IL-2, no relapse
VICC	PCR	*GNAQ*^{Q209P}	None	Thoracic spine	Brain and cervical spine (local dissemination)	31	M	No response to temozolmide

NGS: next generation sequencing; IL-2: interleukin-2; VICC: Vanderbilt Ingram Cancer Center; MDACC: MD Anderson Cancer Center; Ipi: ipilimumab; Pembro: pembrolizumab; Nivo: nivolumab

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