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Activating *CYSLTR2* and *PLCB4* mutations in primary leptomeningeal melanocytic tumors

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Melanocytic tumors arising in the central nervous system are also known as primary leptomeningeal melanocytic tumors (PLMTs). They frequently show a benign behavior and are designated as ‘melanocytomas’ in the World Health Organization classification of central nervous system tumors (Brat et al., 2016). Distinction of PLMTs from melanomas metastatic to the central nervous system can be challenging.

Recent studies have demonstrated that PLMTs have a very characteristic genetic profile, frequently harboring *GNAQ* and *GNA11* mutations (Gessi et al., 2014, Koelsche et al., 2015,

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Kusters-Vandevelde et al., 2010a, Kusters-Vandevelde et al., 2015, Kusters-Vandevelde et al., 2010b, Murali et al., 2012). These mutations are very rare in cutaneous melanoma (Cancer Genome Atlas, 2015), but frequent in uveal melanoma (Van Raamsdonk et al., 2009, Van Raamsdonk et al., 2010). The close molecular relationship between PMLTs and uveal melanomas was further demonstrated by the finding that *EIF1AX, SF3B1* and *BAP1* mutations, previously identified in uveal melanomas, can also occur in PMLT (Kusters-Vandevelde et al., 2016, van de Nes et al., 2016a, van de Nes et al., 2016b). Similar to uveal melanomas, a proportion of PLMTs are not found to harbor activating mutations in *GNAQ* or *GNA11*.

Recent studies have identified activating *CYSLTR2* and *PLCB4* mutations in uveal melanomas, occurring at the L129 and D630 hotspots, respectively (Johansson et al., 2016, Moore et al., 2016). *CYSLTR2* mutations have also been detected in blue nevi (Moller et al., 2016). These mutations always occurred in tumors lacking *GNAQ* and *GNA11* mutations, and have not yet been reported in cutaneous melanomas (Cancer Genome Atlas, 2015). To determine whether *CYSLTR2* and *PLCB4* mutations also occur in PLMTs, we analyzed our previously published cohort of tumors (van de Nes et al., 2016a, van de Nes et al., 2016b), using a next-generation sequencing gene panel covering the mutational hot-spots in *CYSLTR2* and *PLCB4* as well as other gene mutations reported in uveal melanoma (described in Supplemental Material and previously reported (Moller et al., 2016)).

In 19 PLMTs, we found two tumors with activating L129Q (c.386T>A) mutations in *CYSLTR2* and one tumor harboring an activating D630Y (c.1888G>T) mutation in *PLCB4* (Figure 1, Table 1). These mutations were identified at the same hotspots previously described in uveal melanoma and predicted to be activating (Johansson et al., 2016, Moore et al., 2016). In our PLMT cohort, *CYSLTR2* and *PLCB4* mutations were found to be mutually exclusive of *GNAQ* and *GNA11* mutations. Together, mutations in *GNAQ, GNA11, CYSLTR2* or *PLCB4* were identified in 18/19 (94.7%) PLMTs.

The hotspot mutations in *GNAQ* and *GNA11* (R183 and Q209), *PLCB4* (D630) and *CYSLTR2* (L129) result in activation of the corresponding proteins and increased stimulation of a common downstream signaling pathway (Johansson et al., 2016, Moore et al., 2016, Van Raamsdonk et al., 2009, Van Raamsdonk et al., 2010). *CYSLTR2* codes for the CYSLTR2 receptor, a seven transmembrane receptor which signals through the products of the *GNAQ* and *GNA11* genes, the highly homologous heterotrimeric G proteins Gαq and Gα11. Gαq and Gα11 directly activate the *PLCB4* gene product, phospholipase-C β4, which hydrolyses PIP2 (phosphatidylinositol 4,5-bisphosphate) releasing DAG (diacylglyerol) and IP3 (inositol-1,4,5-trisphosphate), subsequently releasing Ca+ (Suh et al., 2008, Waldo et al., 2010). The similar functional consequences of these gene mutations is demonstrated by the occurrence of these mutations in a mutually exclusive fashion.

The increased coverage and sensitivity of our sequencing assay identified multiple additional mutations in other genes, including *EIF1AX* (Figure 1), as was recently reported (Kusters-Vandevelde et al., 2016). One *EIF1AX* mutation (R13C, c.37C>T) was identified in a tumor that also harbored an activating *CYSLTR2* L129Q mutation (Supplemental Figure 1). In addition to a known *SF3B1* R625H (c.1874G>A) and inactivating *BAP1* R60* (c.178C>T)

mutation (van de Nes et al., 2016a, van de Nes et al., 2016b), our targeted next-generation sequencing approach detected a *BAP1* E31del (c.91_93delGAG) mutation in the PLMT sample also harboring a *PLCB4* D630Y mutation (Supplemental Figure 1). The functional relevance of this *BAP1* in-frame one amino acid deletion is unclear. BAP1 immunohistochemistry showed retained nuclear protein expression (Supplemental Figure 2). However, copy number analysis (Supplemental Figure 3) demonstrated loss of chromosome 3 including the other wild-type *BAP1* allele which means the mutation would be highly relevant if it resulted in loss or impairment of protein function. The relevance of *EIF1AX, SF3B1* and *BAP1* mutations in terms of clinical behavior and prognosis needs to be fully elucidated in future larger studies.

In summary, our study demonstrates the occurrence of activating *CYSLTR2* or *PLCB4* mutations in PLMTs lacking *GNAQ* and *GNA11* mutations. Activating mutations in these genes are exceedingly rare in other melanomas, with the exception of uveal melanomas. The diagnosis of a PLMT requires exclusion of a more frequently occurring CNS metastasis of a non-CNS melanoma (most commonly of cutaneous origin). Presence of a *PLCB4, CYSLTR2, GNAQ* or *GNA11* mutation is strong evidence in favor of a PLMT, but a rare CNS uveal melanoma metastasis should also be considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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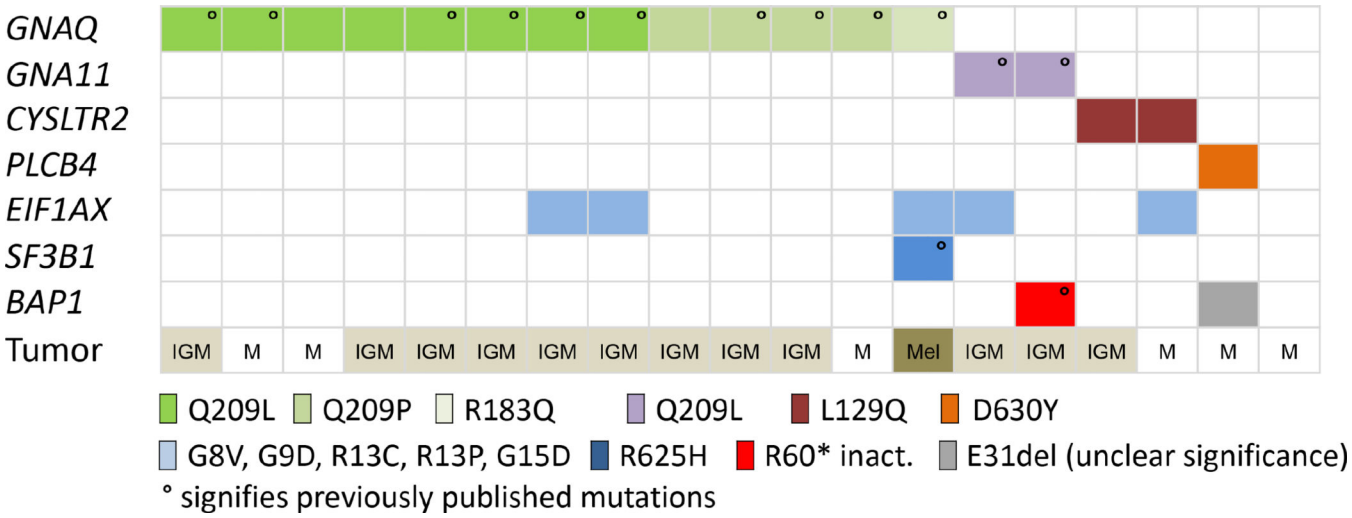

Figure 1. Distribution of mutations in primary leptomeningeal melanocytic tumors

Distribution of mutations identified in primary leptomeningeal melanocytic tumors (PLMT). The histological diagnosis is demonstrated in the lowest row (M = melanocytoma, IGM = intermediate grade melanocytoma, Mel = primary leptomeningeal melanoma). ° signifies previously pu blished mutations (van de Nes et al., 2016a, van de Nes et al., 2016b)

Table 1

Clinicopathological and mutation information of primary leptomeningeal melanocytic tumors

Nr.	Age	Sex	Location	Diag.	Sample	*GNAQ*	*GNA11*	*PLCB4*	*CYSLTR2*	*EIF1AX*	*SF3B1*	*BAP1*	Status	FU
1	46	f	cavernous sinus	M	P	-	-	-	-	-	-	-	NK	NK
2	48	f	spinal	M	P	Q209L°	-	-	-	-	-	-	NK	NK
3	41	f	frontal	M	P	Q209P°	-	-	-	-	-	-	NK	NK
4	73	m	spinal C2-3	M	P	Q209L	-	-	-	-	-	-	NK	NK
5	73	f	temporal right	M	P	-	-	D630Y	-	-	-	E31del	NK	NK
6	77	f	spinal T7-8	M	P	-	-	-	L129Q	R13C	-	-	NK	NK
7	34	m	spinal T10-12	IGM	P	Q209P	-	-	-	-	-	-	A	7y
8	44	m	spinal C1	IGM	P	Q209L°	-	-	-	-	-	-	A	5y
9	79	m	spinal T4-5	IGM	R	Q209L°	-	-	-	-	-	-	NK	NK
10	35	f	spinal C5-6	IGM	P	Q209P°	-	-	-	-	-	-	A	8y
11	65	m	spinal C3	IGM	P	Q209L°	-	-	-	G8V	-	-	A	3y
12	69	f	spinal T8	IGM	P	Q209L°	-	-	-	G9D	-	-	NK	NK
13	64	m	spinal C5	IGM	P	Q209L°	-	-	-	-	-	-	NK	NK
14	NK	NK	NK	IGM	P	Q209L	-	-	-	-	-	-	NK	NK
15	20	m	frontal	IGM	P	Q209P°	-	-	-	-	-	-	A, R#	5y
16	67	m	spinal T9-10	IGM	P	-	-	-	L129Q	-	-	-	NK	NK
17	48	f	spinal T8-9	IGM	R	-	Q209L°	-	-	R13P	-	-	A	6y
18	78	f	tentorium	IGM	P	-	Q209L°	-	-	-	-	R60*°	R§	1y
19	66	m	brain	Mel	P	R183Q°	-	-	-	G15D	R625H°	-	D	1y

green = activating mutations; blue = mutations expected to alter protein function; red = loss of function mutation; grey = mutation of unclear functional significances; Nr. = number; Diag. = diagnosis; m = male; f = female; NK = not known; P = primary; R = recurrence; M = melanocytoma; IGM = intermediate grade melanocytoma; Mel = melanoma; del. = deletion; FU = follow-up period; NK = not known; A = alive; R# = recurred after 3 years (mutation profile was identical to the primary); R§ = recurred, then lost to follow-up; D = dead (disease related)

° signifies mutations previously published (van de Nes et al., 2016a, van de Nes et al., 2016b)