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B RIEF   R EPORT

Identification of a Novel *BRCA1* Alteration in Recurrent Melanocytoma Resulting in Increased Proliferation

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Abstract

Primary meningeal melanocytomas are rare tumors of the central nervous system. Although they are considered benign neoplasms, some reports describe recurrent rates up to 45%. Little is known about their genetic and epigenetic landscape because of their infrequency. Even less has been described about markers with prognostic value. Here we describe a patient who developed a primary meningeal melanocytoma, suffered 3 recurrences in a period of 6 years and died of the tumor. The genetic and epigenetic changes explored confirmed *GNAQ* mutation as an initiating event. We found an epigenetic alteration of *GSTP1*, a feature that has recently been described in meningiomas, from the beginning of the disease. In addition, there was loss of heterozygosity in *BRCA1* beginning in the second recurrence that was linked to an increase in the proliferation index; this suggested a progression pathway similar to the one described in uveal melanomas. These findings underscore the necessity of further research focused on these tumors.

Key Words: *BRCA1*, *GNAQ*, *GSTP1*, Melanocytoma, Meningioma, MLPA, Uveal melanoma.

INTRODUCTION

Primary meningeal melanocytomas (PMMs) are infrequent tumors derived from melanocytes that originate from the neural crest early during embryonic development (1). They are circumscribed neoplasms curable by surgery that are classified as primary melanocytic tumors of the central nervous system (CNS) (2). According to the World Health Organization, malignant transformation of PMMs is rare. The term, "intermediate-grade melanocytic tumor" applies to those tumors with increased mitotic activity and infiltrative growth that fail to meet characteristics of malignant melanoma (2, 3). However, there are reports of aggressive behavior in cases diagnosed as PMM that did not show those intermediate characteristics (1, 4, 5); in fact, some reports describe recurrent rates up to 45% of PMMs, particularly when the follow-up period is about 5 years (1, 3, 6–12).

The genetics of PMM are not well defined. The most common event is the mutually exclusive presence of *GNAQ* or *GNA11* mutations (1, 13–17). Other changes, such as mutations in *BAP1* and losses on 3p21 (14, 17, 18) or mutations in *SF3B1* and *EIF1AX* (19), have been described in these tumors but their possible prognostic roles are controversial. The paradoxical patient outcomes described in the literature and the limited number of genetic studies emphasizes the need to improve the genetic characterization of PMM for better therapeutic management of affected patients.

We studied a patient with a PMM and 3 recurrences that led to a fatal outcome. To the best of our knowledge, this is the first morphological and molecular analysis of a melanocytoma with more than 2 recurrences that also presented with leptomeningeal seeding. This unexpected outcome allowed us to characterize the genetic changes occurred over time as the tumor progressed.

Clinical Summary

A 46-year-old woman was admitted for facial dysesthesia spanning 5 months. Physical examination and anamnesis revealed no other features of interest. Magnetic resonance imaging (MRI) revealed a $29 \times 22 \times 24$ mm mass at the cerebellopontine angle (Fig. 1A). The lesion showed homogeneous enhancement following the administration of gadolinium being hyperintense on T1 and hypointense on T2. The patient underwent a radical craniotomy for removal of the tumor. The surgical removal of the original tumor (OT) was macroscopically complete and brain invasion was not observed. Afterwards, she presented with a left hemiparesis, dysarthria and
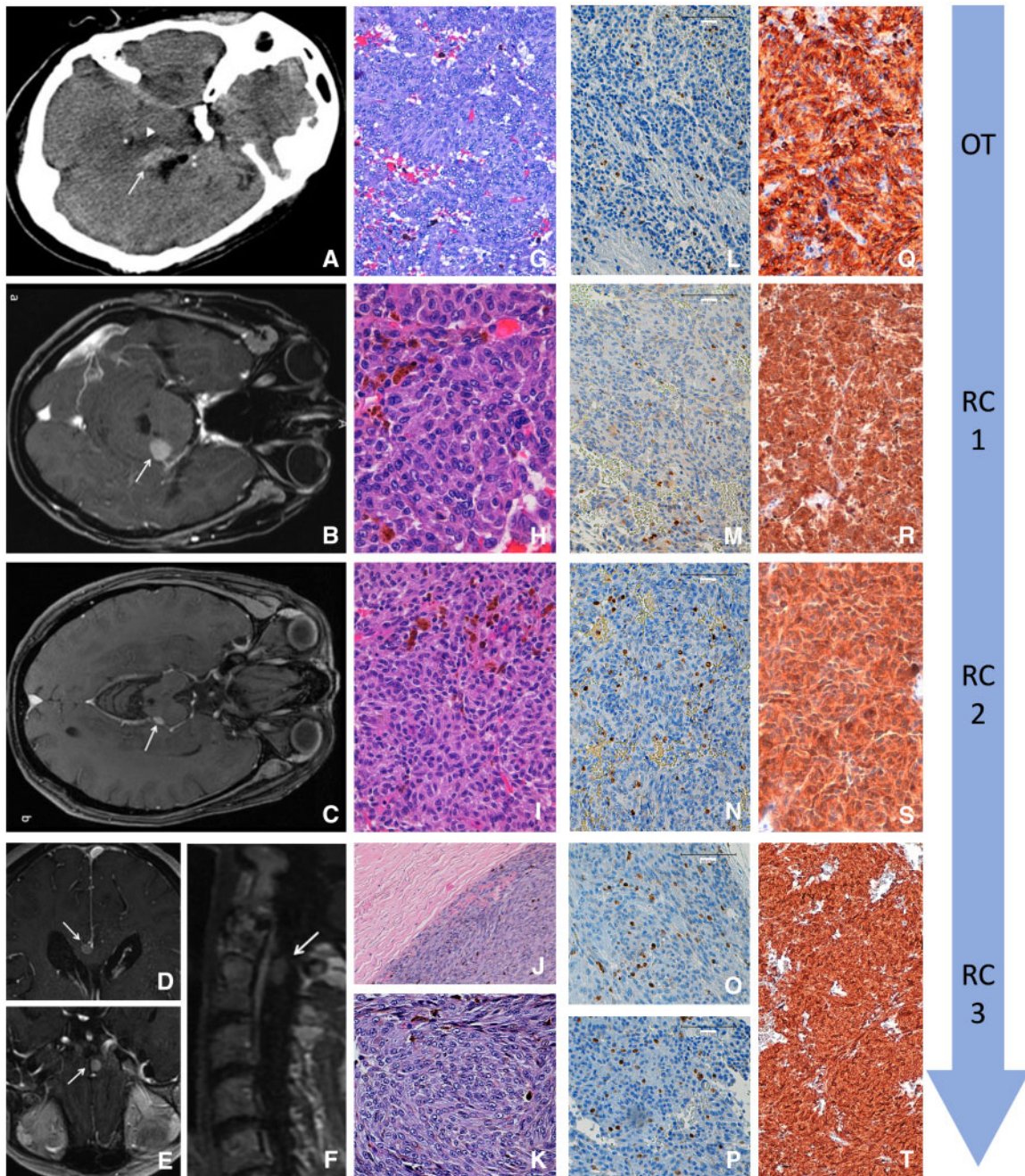
FIGURE 1. Recurrent meningeal melanocytoma. Radiological and histopathological patterns of the OT and recurrences. Radiological patterns. CT scan for postsurgical treatment of the OT showed a residual hemorrhage (arrow) at the cerebellopontine angle (**A**). MRI revealed a local recurrence (RC1) at the cerebellopontine angle after 10 months (**B**). After 53 months, MRI showed, in addition to the local recurrence, a second lesion (arrow; RC2) in the left ambient cistern (**C**). Seventy-two months after diagnosis, a local recurrence and meningeal metastasis were detected by MRI affecting even the cervical area of the spinal cord (**D–F**).Histopathological characteristics. All tumor samples showed a solid- and cord-like growth pattern, with little nuclear polymorphism and a progressive increase in cell density. Little differences were found between the OT (**G**, 20×) and both, RC1 (**H**, 63×) and RC2 (**I**, 40×). RC3 infiltrated the dura mater (**J**, 10×) and showed a more spindled cytoplasmic morphology (**K**, 40×). Ki-67 index was low in the OT (**L**, 20×) and RC1 (**M**, 20×) but increased in RC2 (**N**, 20×) and RC3 (**O, P**, both at 20×); Cytoplasmic immunostaining for Melan-A was seen in the neoplastic cells of the OT (**Q**, 20×), RC1 (**R**, 10×), RC2 (**S**, 20×), and RC3 (**T**, 10×). Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; OT, original tumor; RC, recurrence.

TABLE. Clinical, Immunohistochemical, and Molecular Summary

	OT	RC1	RC2	RC3
Clinical				
Time after diagnosis	NA	10 months	53 months	72 months
Treatment	S	S	S	S
	None	CH1	CH2	PC
Immunohistochemistry				
Vimentin	+	+	+	+
S100	+	+	+	+
HMB45	+	+	+	+
Melan-A	+	+	+	+
EMA	−	−	−Meninges +	−Meninges +
Ki-67 index	1%	2%	5%	5%
Molecular analysis				
BRAF	*Wt*	*Wt*	*Wt*	*Wt*
NRAS	*Wt*	*Wt*	*Wt*	*Wt*
KIT	*Wt*	*Na*	*Wt*	*Wt*
GNAQ	p.Gln209Leu	p.Gln209Leu	p.Gln209Leu	p.Gln209Leu
GSTP1	Met	Met	Met	Na
BRCA1	*Normal*	*Normal*	LOH	LOH

AD, after diagnosis; NA, not applicable; CH1, chemotherapy (fotemustine); CH2, chemotherapy (temozolomide); +, positive immunostaining; −, negative immunostaining; LOH, loss of heterozygosity; Met, methylation; Na, no available; OT, original tumor; RC, recurrence; PC, palliative care; S, surgery; Wt, wild-type

diplopia but no remaining lesion was observed. Ten months later, MRI for disease control revealed a $14 \times 10 \times 12$ mm local recurrence (RC1) that showed similar features on T1 and T2 imaging (Fig. 1B). Thus, a second craniotomy was performed and this was followed by chemotherapy with fotemustine. Despite these efforts, 53 months after diagnosis, MRI revealed a < 1 cm^3 neoplasm in the left ambient cistern (RC2) that also showed the characteristic PMM features of T1-hyperintensity and T2-hypointensity (Fig. 1C). These were found to be a group of small and hard lesions that were growing and were attached to the dura mater. The RC2 could represent a first step towards meningeal dissemination. Cranial nerves were preserved but melanotic pigment was observed on the acoustic nerve. In an attempt to control the disease, treatment with temozolomide was given. However, 72 months after diagnosis, a MRI showed a third recurrence (RC3) that was characterized by many nodular lesions in the cervical area of the spinal cord indicating leptomeningeal spread (Fig. 1D–F). After surgery, because of the refractoriness to medical treatment, the impossibility of a complete resection of the numerous lesions and the initiation of neurological impairment, the patient received palliative treatment and died after 7 years of progressive disease.

MATERIALS AND METHODS

After surgery, tumor specimens were fixed in neutral-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for diagnosis (2). Mitotic figures were counted in 10 high-power fields (HPFs). Immunostaining of different sections was carried out using the avidin–biotin peroxidase method and antibodies directed against vimentin, S100, HMB45, Melan-A, EMA, and Ki-67/MIB1

(all from Dako, Glostrup, Denmark). Proliferation index by Ki-67 assessment was determined in 2 different slides from each sample, exploring 4 HPFs randomly selected in each slide. Fluorescence in situ hybridization (FISH) for chromosome 22 was performed (probe from Vysis, Abbot Scientific, Madrid, Spain), and observed with a Leica LAS AF photomicroscope. DNA was extracted using QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA). The differential diagnosis with metastatic melanocytic tumors originating outside of the CNS included sequencing of *GNAQ, BRAF, NRAS*, and *C-KIT* genes. In addition, copy number variations/methylation status of a set of tumor suppressor genes and oncogenes were analyzed by methylation-specific multiplex ligation-dependent probe amplification ([MS-LPA] ME001-C1, MRC Holland, Amsterdam, The Netherlands).

RESULTS

Histopathological examination of the OT revealed a neoplasm with high cellularity composed by slightly spindled tumor cells with oval nuclei and scarce, irregular and homogeneous cytoplasms containing melanin. Neoplastic cells formed solid nests and showed a vasocentric pattern of growth with some pseudopapillary structures. The vascular net was wide and the tissue showed some hemorrhagic areas. Mitoses were infrequent and isolated (<1/10 HPFs). Ki-67 labeling-index was 1%. Immunohistochemical staining was positive for vimentin, Melan-A, HMB-45, and S100 (Fig. 1G–I). Together, these findings pointed to the diagnosis of PMM (Table). The OT was wild-type for all the markers assayed except for *GNAQ* in which a Gln209Leu mutation was found (Fig. 2). FISH for chromosome 22 showed disomy and MLPA showed an unexpected hypermethylation of *GSTP1*. A sum-
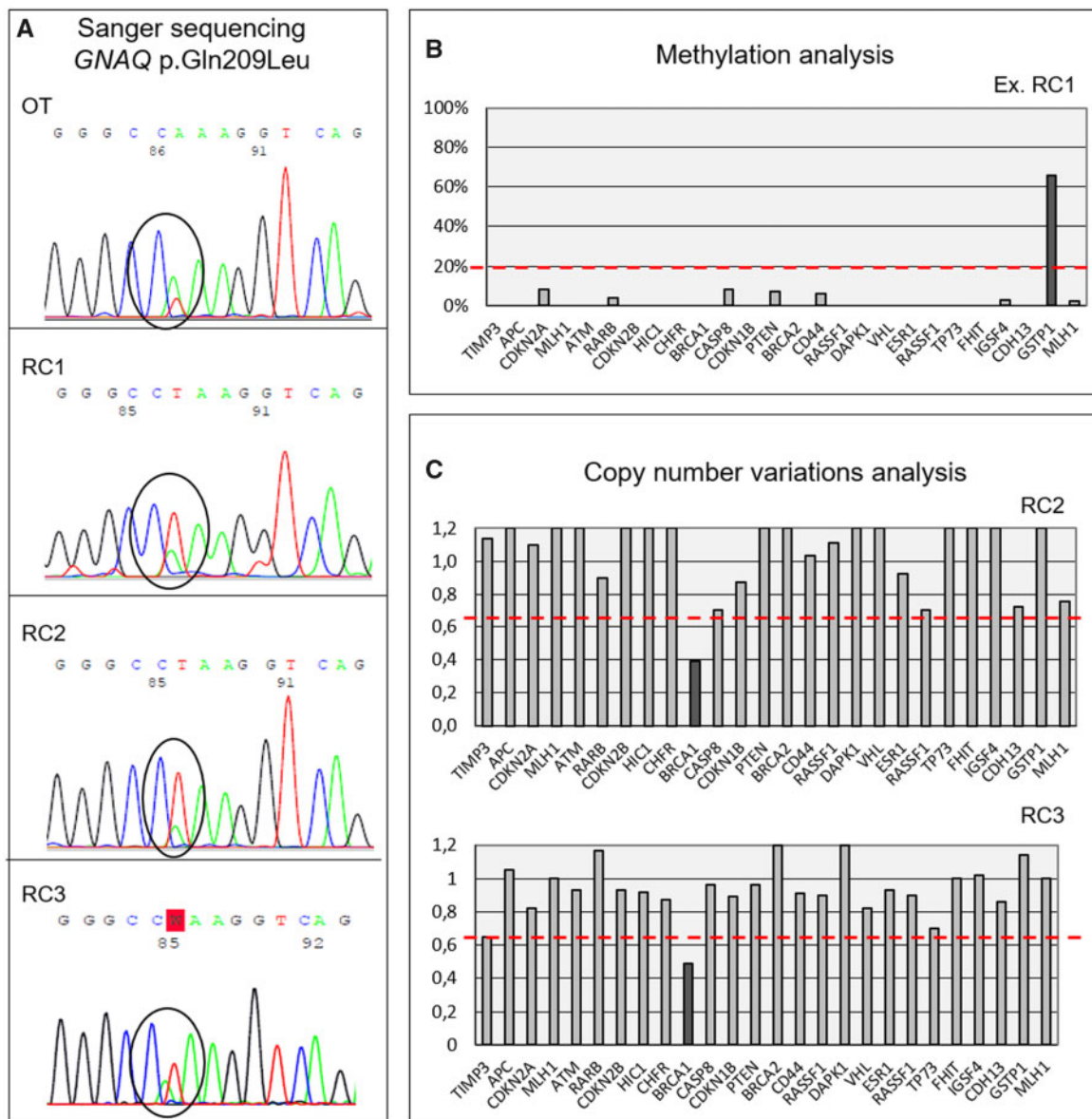
FIGURE 2. Molecular analysis. Sanger sequencing revealed that all samples were homogeneously affected for *GNAQ* mutation p. Gln209Leu (**A**). MS-MLPA for assessing the methylation status of tumor suppressor genes showed marked hypermethylation of *GSTP1*; the example shown corresponds to RC1 (**B**). MLPA for copy-number variation analysis revealed a loss of heterozygosity in *BRCA1* in RC2 and RC3 (**C**). Abbreviations: MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; OT, original tumor; RC, recurrences.

mary of the genetic characteristics found is shown in the Table.

The morphological features of the first recurrence (RC1) were similar to those in the OT but there were more prominent nucleoli and the presence of melanophages. We found positivity for the same immunohistochemical markers, a low proliferation rate (Fig. 1J–L), and the same molecular profile (Table).

The second recurrent tumor (RC2) displayed some changes. The histological study confirmed dura mater attachment. The cellularity was also high and a vasocentric pattern, more spindled-cell shapes, aggregations forming nests and the

presence of melanophages were observed. The mitosis count was low (<1/10 HPFs) but, interestingly, in this sample Ki-67 labeling-index increased to 5%, suggesting more rapid growth. The remaining immunohistochemical markers were the same as those of the previous samples (Fig. 1M–O). In addition to the *GNAQ* mutation and the *GSTP1* hypermethylation found in the previous samples, we detected a loss of heterozygosity (LOH) of *BRCA1* (Fig. 2 and Table).

The RC3 showed morphological features that were similar to the previous samples although the tumor cells showed less defined cytoplasm and a great amount of melanotic pigment. The proliferation study showed <1 mitosis/10 HPFs but

the proliferation index remained in 5% of Ki-67 (Fig. 1P–T); there was also the *BRCA1* LOH (Table).

DISCUSSION

PMMs presumably arise from leptomeningeal melanocytes (2); their neural-crest derivation and migration justify the naming and classification of these tumors (1, 20). However, their consideration as benign neoplasms seems to require better subclassification. Here, we present a patient who suffered multiple recurrences and leptomeningeal dissemination of a PMM. With respect to the differential diagnosis, it is important to emphasize that melanomas usually show adjacent T2-hyperintense areas due to vasogenic edema (2); therefore, no transformation into a different entity occurred during the progression of the disease in our patient. MRI showed small, nodular, and physically close lesions with similar histological characteristics in the different samples. Together, these findings support the common origin of all of the patient's tumors.

Genetic analysis helps to distinguish among melanocytic tumors and metastasis from other locations although extensive genetic descriptions of PMM are scarce (1, 2). *BRAF, NRAS, HRAS, KRAS*, and *KIT* have been explored in PMMs (7, 13–15, 21), but only occasional findings of *BRAF* V600E and *NRAS* mutations have been reported (14, 21). The *RAS/KIT* wild-type genotype findings agree with our results in that the Gln209Leu mutation in *GNAQ* was a common change in all the samples despite the different location of the recurrences (Fig. 2 and Table). No concrete epigenetic change has been described in any previous reports on PMM other than a study by Koelsche *et al* (14). Those authors identified distinctive methylation profiles among PMMs. They did not describe the genes that were specially implicated in each group but enhanced *GSTP1* methylation, as we found in our patient from the beginning of the disease, could represent a characteristic change in these tumors. This unexpected finding has been recently described on meningioma (22) and represents an epigenetic phenomenon shared by these two distinct tumor types both of which originate from the meninges. In contrast, however, no 22q loss was found by FISH in any of the samples. PMMs also share genetic characteristics with noncutaneous melanomas such as uveal melanoma. In uveal melanoma, *GNAQ* or *GNA11* mutations arise early and inactivation of *BAP1* occurs in a later phase of the oncogenic process (2, 19). *BAP1* encodes the *BRCA1*-associated protein 1, which binds to *BRCA1* and acts as a tumor suppressor, although its role is still being investigated. Surprisingly, we found LOH in *BRCA1* during the leptomeningeal spread (R2 and R3; Fig. 2) accompanied by a histological feature of increased aggressiveness. This striking observation points to a PMM that progressed more similarly to uveal melanoma than to meningiomas. Although the relationship between *BAP1* and *BRCA1* is not well understood, our finding underlines that they might have related roles. In addition, drug resistance to fotemustine and temozolomide could be achieved in part by this genetic change (23).

In summary, we present a tumor that recurred locally and begun a process of dissemination with genetic and epigenetic alterations that have not been previously described in PMM. The coincidence with meningioma of an epigenetic change from the early state of the tumor parallels the unexpected progression of the disease in pattern similar to that of UM. This progression adds to the dilemma of classification based only on histopathologic features. Further research is required to improve the characterization of the genetic underpinnings of PMM. Whether *GSTP1* epigenetic alteration is an initiation event needs to be confirmed in other patients. The elucidation of the potential role of *BRCA1* in progression of PMM and the identification of additional markers of aggressiveness are needed to improve the clinical management of PMM patients.

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