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Research Article

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Primary Choroidal Melanoma with Divergent Neuroendocrine Differentiation

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Abstract

Background: Despite well-defined histopathological characteristics, melanoma has been reported to have different histological and immunohistochemical variations that can lead to diagnostic controversy. Neuroendocrine divergent differentiation has been demonstrated previously in skin and metastatic melanoma, and also in metastatic tumors to the choroid, but not in primary choroidal melanoma.

Aim: To report the clinical, histopathological, immunohistochemical and ultrastructural characteristics of four cases of primary choroidal melanoma with neuroendocrine differentiation.

Methods and Results: A retrospective case series analysis was conducted for all patients with choroidal melanoma with confirmed histopathological features of neuroendocrine differentiation, evaluated at the Laboratory of Ophthalmic Research and Visual Sciences. Four cases were identified, two male and two female. Preoperative clinical diagnosis was choroidal melanoma for all cases. Atypical small-cell morphology distributed in a pattern forming an organoid, perivascular or nested architecture on light microscopy was found in all cases, which prompted further evaluation with neuroendocrine markers. Immunohistochemistry demonstrated positivity in at least three melanocytic and three neuroendocrine markers for each case. Hi-resolution optic microscopy showed granules corresponding to intracytoplasmic neurosecretory and melanin granules in the same cells, confirming the diagnosis.

Conclusions: In this study we provide evidence suggestive of divergent neuroendocrine differentiation in primary choroidal melanoma.

Keywords

Choroidal melanoma; Immunohistochemistry; Neuroendocrine differentiation

Introduction

Uveal melanoma is the most common primary intraocular malignancy in adults and one of the most studied malignancies in the eye. Despite well-defined histopathological characteristics, melanoma has been reported to have different histological and immunohistochemical variations that can lead to diagnostic controversy [1]. Divergent differentiation in melanoma has been defined as the development of morphologically, immunohistochemically and/or ultrastructurally recognizable non-melanotic cell or tissue components [2]. Neuroendocrine divergent differentiation has been demonstrated previously in skin and metastatic melanoma [3,4], and also in metastatic tumors to the choroid [5]. We report the clinical, histopathological, immunohistochemical and ultrastructural characteristics of four cases of primary choroidal melanoma with neuroendocrine differentiation.

Materials and Methods

A retrospective case series analysis was conducted through a chart review of all patients with choroidal melanoma with confirmed histopathological features of neuroendocrine differentiation, evaluated at the Laboratory of Ophthalmic Research and Visual Sciences (LIOCiV), School of Medicine, Buenos Aires University, from 1990 to 2010. Although our study was retrospective, all patients evaluated for choroidal melanoma in the Institute during the specified years underwent a comprehensive prospective ophthalmic examination and a prospective histological analysis when specimens were obtained. Patient data included demographics, ocular and medical history. Tumor specimens were fixed in formalin and sections were studied with hematoxylin and eosin, Masson's trichrome, periodic acid-schiff staining and immunohistochemistry (ICH). The tissue sections were immunoperoxidase-labeled using melanotic and non-melanotic (neuroendocrine) antibodies (Biogenex). Specific melanotic antibodies used included: HMB 45 (super sensitive monoclonal), protein S-100 (high performance polyclonal) and Melan-A (MART monoclonal AM361-5M). Neuroendocrine antibodies used included: chromogranin A (super sensitive monoclonal), synaptophysin (Monoclonal, AM 363-5M), cytokeratin (super sensitive monoclonal), calcitonin (high performance polyclonal) and vasoactive intestinal polypeptide (VIP) (super sensitive polyclonal).

Fifteen sections were contrasted with a fixed-network and another 15 with diaminobenzidine. Both variants were used to determine that there were no alterations caused by the developer. In all cases, vimentin was used as a control marker.

Hi-Resolution Optic Microscopy (HROM) was performed with a Siemens microscope; pellets were fixed in a 0.1 M cacodylate buffer with glutaraldehyde; the thick cuts (1 µm) were stained with toluidine-fuchsin and the ultra-fine cuts with uranyl acetate Reynolds.

Results

Four cases were identified, two were male and two female. Mean age was 45 years (range 38-52 years). No previous ocular or medical history of relevance was noted. Preoperative clinical diagnosis was choroidal melanoma for all cases. In three cases the melanoma was in the macular area and in one case in the infero-temporal quadrant. All cases showed the presence of localized exudative retinal detachment (RD). All tumors were pigmented and brown to dark brown in

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color. The mean tumor apical thickness was 11.5 mm (9.2-13 mm) and the mean largest base diameter was 13.6 mm (6.1–17.1 mm). There were no clinical or ancillary testing findings suggestive of extrascleral extension. Treatment included enucleation in three cases and a lamellar trans-scleral choroidectomy plus Iodine 125 plaque brachytherapy in one case. No local recurrence or metastases were observed at a mean follow-up of 6.1 years (5–7 years).

Gross examination of enucleation specimens showed two dome-shaped and one mushroom-shaped choroidal pigmented mass. The specimen obtained from the choroidectomy showed a dome-shaped mass with adequate free margins. Histology confirmed the absence of scleral invasion; no vessel dissemination was found either.

Atypical small-cell morphology distributed in a pattern forming an organoid, perivascular or nested architecture on light microscopy was found in all cases. This microscopic feature is typical of tumors that present neuroendocrine differentiation, which prompted further evaluation with neuroendocrine markers.

All cases were positive for the three melanotic IHC markers used in this study: HMB-45 (Figure 1a), S100 protein and Melan-A. All cases were also positive for two of the non-melanotic (neuroendocrine) markers: chromogranin and synaptophysin (Figures 1b and 1c). In cases 2 and 3 cytology showed a visible nucleolus and cytoplasm with granular pigmentary structures. Case 2 was also positive for cytokeratin, and case 3 was also positive for VIP, both accepted neuroendocrine markers. Cases 1 and 4 showed low structures being positive for neuroendocrine activity using IHC with chromogranin, synaptophysin, calcitonin (Figure 1d) and VIP. Markedly positive granules are directly related to the presence of melanosomes detectable with antibodies to S-100, HMB 45 and Melan-A. HROM demonstrated in all cases that the granules corresponded to intracytoplasmic neurosecretory and melanin granules in the same cells (Figures 1e and 1f).

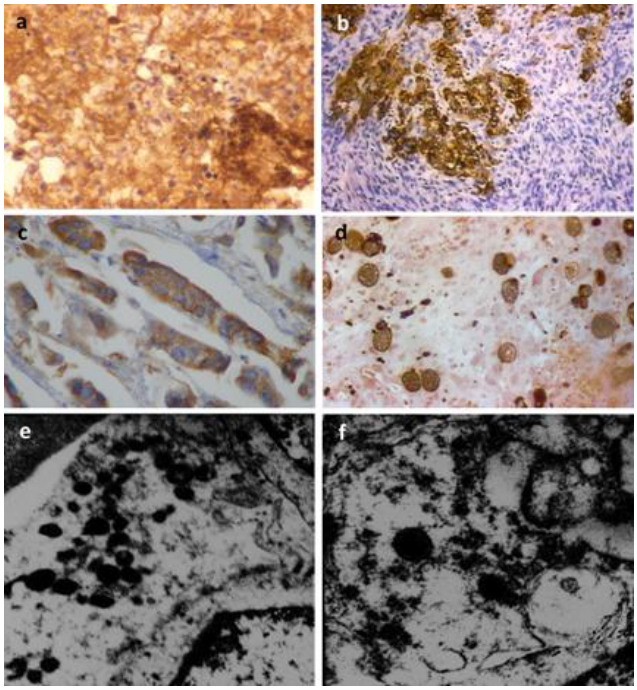

Figure 1: Description of Choroidal Melanoma.

a-d: Immunohistochemistry of choroidal melanoma.
a: HMB45. The stain is focal, strong and cytoplasmic (40x).
b: Chromogranin. The stain is diffuse and membranous (20x).
c: Synaptophysin. The stain is stronger in tumoral cluster cells (60x).
d: Calcitonin. This is more specific in isolated cells with strong membranous and soft cytoplasmic stain (60x).
e,f: Hi-resolution optical microscopy of choroidal melanoma with neuroendocrine differentiation. Nests of melanosomes alternated with capsulated neuroendocrine granules are shown.
e: 6000x.
f: 9000x.

Discussion

Mucocutaneous melanoma has been demonstrated to have divergent differentiation into fibroblastic/myofibroblastic, Schwannian and perineural, smooth muscle, rhabdomyosarcomatous, osteocartilaginous, ganglionic and ganglioneuroblastic, and neuroendocrine differentiation [2]. Both mucocutaneous and uveal melanoma has been partially demonstrated with neuroendocrine differentiation by many authors [6-14]. Definitive histological and immunohistochemical features have been described previously by Eyden et al. as: at least three accepted melanocytic markers and three accepted neuroendocrine markers [3]. The most accepted melanocytic markers include HMB-45, S100 protein, Melan-A and tyrosinase. There are several neuroendocrine markers; chromogranin and synaptophysin are the most commonly used. Chromogranin has been described as "the single most specific generic marker of neuroendocrine differentiation in general use" [15]. The combination of these two broad-spectrum markers improves greatly the specificity and sensitivity for neuroendocrine tumors [16]. Other accepted neuroendocrine markers include neuron-specific enolase (NSE), neurofilament protein, proconvertases, neuron cell adhesion molecule (NCAM), cytokeratin, calcitonin and VIP, among others [17,18].

There are only two reports of definitive neuroendocrine differentiation of melanoma, including 4 cases in total [3,4]. Of those cases, two were primary cutaneous melanoma, one primary nasal mucosal melanoma and one primary malignant tumor of the lung with both neuroendocrine and melanoma differentiation. All three patients diagnosed as primary melanomas simultaneously presented or subsequently developed metastatic disease. All of our cases were primary malignancies of the choroid; baseline and subsequent systemic evaluation at a minimum follow-up of 5 years on all cases demonstrated no other primary or metastatic malignancies.

In our study, IHC analysis met the criteria for neuroendocrine divergent differentiation of malignant melanoma in all cases. Specimens were positive for S-100, HMB-45 and Melan-A confirming the clinical diagnosis of choroidal melanoma. Positivity for chromogranin A and synaptophysin was present in all cases, demonstrating the neuroendocrine component. All cases were positive for at least one more neuroendocrine marker, confirming the neuroendocrine differentiation. Furthermore, ultrastructural analysis by electron microscopy supported the diagnosis in all cases.

The most important differential diagnosis is a neuroendocrine tumor metastatic to the uvea as previously reported [5,19,20]. Most of those patients have a history of a non-ocular neuroendocrine neoplasm, or the baseline and follow-up systemic evaluations readily reveal the primary tumor. Neuroendocrine tumors metastatic to the choroid present a diagnostic challenge since these tumors can adopt

Table 1: Differentiating features of choroidal melanoma with NE differentiation. Considering: 10 Hi-Power Field (HPF) 40x: + 1-3 positive HPF, ++ 4-6 positive HPF, +++ 7-10 positive HPF, -0 positive HPF.

Type	Previous Oncological History	Light Microscopy	Immunohistochemistry	Electronic Microscopy
Uveal Melanoma	No	Spindle, epithelioid or mixed cell type	Chromogranin (-) S-100 (+++) HMB45 (+++) Melan-A (+++) Calcitonin (-) VIP (-)	Melanin Granules
Carcinoid metastatic to the uvea	Yes	Insular, fascicular or perivascular pattern. Intracytoplasmic granules	Chromogranin (+++) S-100 (+ or -) HMB45 (-) Melan-A (-) Calcitonin (+) VIP (+)	Intracytoplasmic neurosecretory granules
Neuroendocrine Uveal Melanoma	No	Spindle, epithelioid or Mixed cell type with pattern insular, fascicular or perivascular. Intracytoplasmic granules	Chromogranin (+++) S-100 (+++) HMB45 (+++) Melan-A (+++) Calcitonin (+) VIP (+)	Melanin and Intracytoplasmic neurosecretory granules in neoplasic cells

different shapes, present different coloration and even appear as a pigmented mass [5], simulating other metastatic tumors and also primary choroidal melanoma. Differential diagnosis is made based on IHC specific markers and confirmed by electron microscopy (Table 1). These tumors are negative for melanoma specific markers on ICH and the electron microscopy fails to demonstrate melanin granules along with neuroendocrine granules in the same clone of cells.

Neuroendocrine differentiation of choroidal melanoma is a histological and IHC finding with possible prognostic implications. All cases presented clinically with a large choroidal mass and therefore the treatment included enucleation in three cases and choroidectomy with subsequent brachytherapy in one case, instead of brachytherapy alone which is reserved for medium-sized tumors. Although these were large tumors, no case has developed metastases at a minimum follow-up of 5 years. It seems to appear that this group of patients has a favorable systemic prognosis regarding metastatic spread. It is important to note that no histological evidence of scleral invasion or vessel dissemination was found. This may contribute to the favorable course these patients are exhibiting. However, we acknowledge that no clinical conclusions can be made from this small series.

Future studies will help identify new histological and functional variants for uveal melanoma, ensuring a more analytical classification that is perhaps more complex, but leads to a better assessment of the prognosis and progression of pigmentary malignant tumors of the uveal tract.

In conclusion, we present four cases clinically diagnosed as choroidal melanoma. In all cases the histological characteristics of the specimens prompted suspicion of neuroendocrine differentiation. This was supported by IHC and definitive diagnosis was made by ultrastructural analysis. In this study we provide evidence suggestive of divergent neuroendocrine differentiation in primary choroidal melanoma.

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