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MELANOCYTIC TUMOUR PATHOLOGY

Satellite in transit metastases in rapidly fatal conjunctival melanoma: implications for angiotropism and extravascular migratory metastasis (description of a murine model for conjunctival melanoma)

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Summary

Little information is currently available concerning loco-regional metastases such as satellite and in transit metastases and their natural history in conjunctival melanoma as compared to cutaneous melanoma. Angiotropism, a marker of extravascular migration of melanoma cells along vascular channels, often appears responsible for microscopic satellite, satellite and in transit metastases development in cutaneous melanoma. In addition, diffuse tissue microscopic satellites are correlated with widespread melanoma dissemination and death. Herein we report rapid conjunctival melanoma progression and a fatal outcome in four of five patients following recurrence as satellite in transit metastases. Five patients aged 31, 60, 63, 56, and 67 years developed primary conjunctival melanoma, histologically characterised by tumour thicknesses of 4, 4, 1.1, 3, and 2 mm. Two or more conjunctival melanomas manifested ulceration, significant mitotic rates, necrosis, angiotropism, and intralesional transformation. The conjunctival melanoma recurred in a matter of months as one or more discrete satellite in transit lesions in the vicinity of the primary melanoma. Histological examination revealed well-defined micronodules containing atypical melanocytes in the subepithelial connective tissue stroma. All lesions were extravascular and most appeared angiotropic. Four of five patients subsequently developed parotid or other loco-regional nodal disease and rapidly ensuing widespread metastases and death. The time course from diagnosis to the demise of the patients averaged about 13 (range 7–20) months. Our findings suggest that satellite in transit metastases constitute an important new risk marker for possible rapid metastatic disease progression and death in patients with conjunctival melanoma. This finding appears to take on even greater significance if such lesions develop rapidly, i.e., in a matter of weeks or months following diagnosis of primary conjunctival melanoma, and if the primary melanoma manifests additional high-risk features. Additional studies are underway in order to further elucidate the mechanism of these metastases.

Key words: Conjunctival melanoma; metastasis; microscopic satellite; in transit metastasis; satellite; angiotropism; extravascular migratory metastasis; melanoma.

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INTRODUCTION

Conjunctival melanoma is rare, and as a result, its biology and natural history remain poorly understood.[1–7] The generation of reliable information about the neoplastic nature of conjunctival melanoma and predictors of clinical outcome are of critical importance. Pertinent clinical and histopathological prognostic factors elaborated for conjunctive melanoma include anatomical site (i.e., bulbar versus non-bulbar conjunctiva); extent of disease (involvement of sclera, orbit, or skin); lesional diameter (e.g., >10 mm versus <10 mm); tumour thickness (e.g., >2 mm versus <2 mm); mitotic rate; cell type; ulceration; and lymphovascular invasion.[1–7]

Since conjunctival melanomas of any size may metastasise, there is a great need for more detailed information about the mechanisms of metastasis in this neoplastic system. As with cutaneous melanomas, conjunctival melanomas are believed to achieve progressive loco-regional spread via lymphatic channels and subsequent more distant dissemination through the blood vascular system. The presence of a rich lymphatic plexus in the substantia propria and caruncle has been proposed as the basis for such loco-regional conjunctival melanoma spread. In any case, however, the mechanisms responsible for metastasis of conjunctival melanoma, as in virtually all neoplastic systems, require much more rigorous investigation.

Mounting evidence now indicates that cutaneous melanoma and other solid tumours may spread to regional and potentially distant sites by an alternative mechanism, a phenomenon termed angiotropism and 'extravascular migratory metastasis' (EVMM).[8–10] A number of investigations have established that angiotropism is an important marker of loco-regional metastases,[8,10–15] such as microscopic satellite,

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satellite, and in transit metastases,[10–15] and a prognostic factor in cutaneous melanoma. With reference to conjunctival melanoma, there appears to be very limited information currently available about comparable metastatic lesions.

Herein we report our experience with satellite in transit metastases in five patients with conjunctival melanoma. Four of these five patients manifested rapid conjunctival melanoma progression and a fatal outcome following recurrence as satellite in transit metastases. We also report the development of a patient-derived xenograft mouse model obtained from a subcutaneous metastasis of conjunctival melanoma from one of these patients. Such models may provide an additional technique for the investigation of the biology of conjunctival melanoma metastasis.[16,17]

MATERIAL AND METHODS

Patient materials

Five patients were referred to the Department of Ophthalmology, Institut Curie, for the management of primary conjunctival melanomas and who subsequently were observed to develop distinctive metastatic lesions in close proximity to their primary melanomas during the period 2003–2015. Clinical, histological, molecular, and clinical outcome data were recorded (Tables 1–4) for each patient. Clinical parameters recorded were as follows: age, gender, anatomical site of the primary melanoma, clinical presentation, and antecedent lesion. Histological characteristics recorded were: Breslow thickness, ulceration, mitotic rate (per mm^2), necrosis, presence of angiotropism, vascular/lymphatic invasion by melanoma, intralesional transformation, and mutational status for *BRAF*, *NRAS*, and *CKIT*. Intralesional transformation refers to cytomorphological heterogeneity within the primary melanoma, in particular, the observation of phenotypically different populations of melanoma cells, suggesting tumour progression (clonal evolution) within the primary melanoma to a presumably more aggressive neoplasm. Attributes of the satellite in transit metastases for each case which were recorded included: the interval from diagnosis of primary melanoma to the development of satellite in transit lesions, anatomical site, number of lesions, dimensions of the lesions, angiotropism/extravascular location of melanoma cells, necrosis, vascular/lymphatic invasion, and principal cell type. Additional information recorded (Table 4) included: initial therapy, systemic therapy, location of regional and distant metastases, clinical outcome and length of follow-up in months.

Angiotropism was defined as previously described:[8,9] (1) clearly recognisable (unequivocal) melanoma cells closely opposed to the abluminal (external) surfaces of the endothelium of microvascular and/or lymphatic channels, either in linear array or in aggregates, (2) the latter occurring in at least one or more foci and (3) no evidence of intravascular or intralymphatic melanoma cells. In order to distinguish angiotropism from apparent non-specific entrapment of microvessels by the main tumoural mass, angiotropism was recorded as present only if it was observed at the peripheries or advancing front of a tumour or in nearby peri-tumoural tissue within 1– 2 mm of the primary tumour.

Genetic analysis

Both human and mouse melanoma samples were examined for *BRAF*, *NRAS* and *CKIT* mutations. Somatic genotype analysis for *BRAF* targeted exon 15, for *NRAS* exons 12, 13, 61, 117 and 146, and for *CKIT* exons 11, 13 and 17. Cytoscan HD (Affymetrix, USA) was utilised to assess chromosomal copy number status in the latter samples. Sequencing of the gene *PTEN* was also performed.

Establishment of the xenograft mouse model

Animals

Healthy adult SCID mice (Charles Rivers Laboratories) aged 6 weeks were used for the study. All mice were maintained under controlled light conditions (12 hour light/dark schedule) with unrestricted access to food and water at the Institute Curie animal facility under pathogen-free conditions. At the conclusion of the experiments the animals were euthanised. Animals were handled in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in Ophthalmology and Research in Vision.

Tumour sampling for research

Informed consent (Case 1) was obtained for the use of tissue samples for experimental research. Fresh tumour samples were provided by the Department of Pathology immediately after surgical removal of a subcutaneous melanoma metastasis from Case 1. The tumour samples were grafted into the interscapular fat pad of SCID mice (Charles River Laboratories) aged 6 weeks (p0 passage). When a tumour reached a 1 cm^3 volume, the mice were sacrificed and the tumours were grafted into other SCID mice (p1 passage).[18] Tumour samples were collected at every passage and frozen in DMSO solution or fixed in AFA for analysis.

RESULTS

Summary of clinical and histological findings

The clinical characteristics of the five patients and the histological findings are summarised in Tables 1 and 2 and shown in Fig. 1–4.

Five men ranged in age from 31 to 67 years with a median of 60 years and mean of 55.4 years. Four of five primary melanomas involved the bulbar conjunctiva, and one lesion the tarsal palpebral conjunctiva (Fig. 1A, 3A, 4A). All five lesions were notable for recent changes in size and Case 5 had experienced diminished visual acuity. A history of antecedent conjunctival naevus was described in three patients, whereas two were noted to have primary acquired melanosis (PAM) at presentation at Institut Curie. The lesions ranged in diameter from 3.5 to 10.0 mm with median of 6.0 mm and mean of 6.5 mm. The patients had no previous history of melanoma or other clearly defined risk factors for melanoma. All patients were treated by 'no touch' surgical excision under general anaesthesia followed by irradiation of the surgical bed by proton beam radiotherapy.

Histologically, all of the lesions demonstrated *in situ* and invasive melanoma components. In general, the lesions exhibited an admixture of enlarged epithelioid, ovoid, or spindled melanocytes, and all lesions were characterised by sheet-like or nodular growth of melanocytes in the substantia propria. Prominent melanophage accumulation was noted in Case 2. None of the lesions showed conventional maturation

Table 1 Primary conjunctival melanomas: clinical features

Patient	Age, years	Gender	Site	Clinical presentation	Antecedent lesion
1	31	M	Temp bulbar	Increasing size	Naevus
2	60	M	Temp bulbar	Increasing size	Naevus
3	63	M	Tarsal	Pigmented lesion	PAM
4	56	M	Temp bulbar	Pigmented lesion	PAM
5	67	M	Temp bulbar	Changing lesion, LOVA	Naevus

LOVA, loss of visual acuity; PAM, primary acquired melanosis; Tarsal, tarsal conjunctiva; Temp bulbar, temporal bulbar conjunctiva.