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In vivo detection of monosomy 3 in eyes with medium-sized uveal melanoma using transscleral fine needle aspiration biopsy

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PURPOSE. *Cytogenetic prognostication of choroidal melanoma, particularly monosomy 3 detections, is limited to enucleated eyes or resected tumors. The authors developed an* in vivo *technique to detect monosomy 3 using transscleral fine needle aspiration biopsy (FNAB).*
METHODS. *Eight eyes with medium-sized choroidal melanoma were included in this prospective study. A 25-gauge transscleral FNAB was performed during surgical procedure for brachytherapy, just before applying the radioactive plaque over the tumor base. Sampled material underwent fluorescence in situ hybridization (FISH) with centromeric probes for chromosome 3. Follow-up was >12 months.*
RESULTS. *Transscleral FNAB yielded sufficient material in 7 of 8 eyes (87.5 %). Five of seven eyes had monosomy 3. No early or late complications were detected.*
CONCLUSIONS. *This study demonstrates that medium choroidal melanomas may be safely sampled by intraoperative transscleral FNAB to detect monosomy 3* in vivo. *(Eur J Ophthalmol 2006; 16: 422-5)*

KEY WORDS. *FNAB, Monosomy 3, Uveal melanoma*

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INTRODUCTION

Posterior uveal melanoma is the most common primary intraocular malignant tumor in adults (1). Although fewer than 2% of affected patients have clinically detectable metastases at presentation, about 50% of all treated patients ultimately die of metastatic disease (2). The average life expectancy after diagnosis of metastasis is about 7 months, because no effective treatment exists for disseminated disease (3). Life prognosis is currently based on clinical or histologic parameters. Because most posterior (choroidal and ciliary body) uveal melanomas are currently treated with radiation, no material is available for cytologic or histologic evaluation. Therefore, most treated patients receive prognostic information based only on clinical characteristics of the tumor (largest basal diameter, thickness, location). Unfortunately, these parameters are unable to accurately characterize patients' prognosis (4).

It has been recently demonstrated that a cytogenetic characteristic of posterior uveal melanoma, namely monosomy 3, is highly predictive of metastatic disease (4-6). This parameter seems a better predictor of metastasis than any other clinical and/or histologic parameter previously reported (5). Monosomy 3 has been detected in enucleated eyes or histologic specimens from resected

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tumors (6-9). Patients treated with irradiation seem to be excluded from this prognostic information.

The aim of this study was to evaluate the feasibility of *in vivo* detection of monosomy 3 in eyes undergoing plaque brachytherapy.

METHODS

This study was performed on patients referred to the Ophthalmic Oncology Unit of our department for plaque brachytherapy of choroidal melanoma, and approved by the IRB of our institution. To be included in this pilot study each patient had the following characteristics: medium-sized choroidal melanoma (thickness >5 mm) without extrascleral extension, free of metastasis and other cancer at enrollment, 21 years or older, and free of other coexisting disease threatening survival for the following 5 years. Each patient signed an informed consent. Eight consecutive patients, with a follow-up longer than 12 months, are included in this report. All patients underwent complete ophthalmologic examination, including A- and B-scan ultrasonography. Liver enzymes and liver ultrasonography were used to check for liver metastatic diffusion at baseline. All patients were scheduled for standard plaque brachytherapy under general anesthesia. During the operation, just before suturing the active plaque, a transscleral fine needle aspiration biopsy (FNAB) of the tumor was performed.

FNAB procedure was performed using a 25 gauge (25 mm in length) spinal needle connected to a 10 cc syringe by a hollow tube. The needle was inserted into the tumor through a 300 µm scleral incision (to avoid excessive pressure when penetrating the eye) (Fig. 1). A double-pass sampling was performed. The scleral incision was sutured and the radioactive plaque immediately placed over the tumor base. A standard dose of 100 Gy was delivered at the tumor apex. Tumor specimens obtained by FNAB were collected in culture medium RPMI 1640 (Euroclone Life Science, Pero-MI, Italy).

All patients underwent a 1-, 3-, and 6-month follow-up ophthalmic examination, and every 6 months thereafter. Follow-up examination included complete eye evaluation and A- and B-scan ultrasonography. Liver enzyme analysis and ultrasonography were performed at 6-month intervals.

The sampled material underwent fluorescence in situ hybridization (FISH). After sedimentation, the material was enzymatically digested with collagenase II (Worthington,

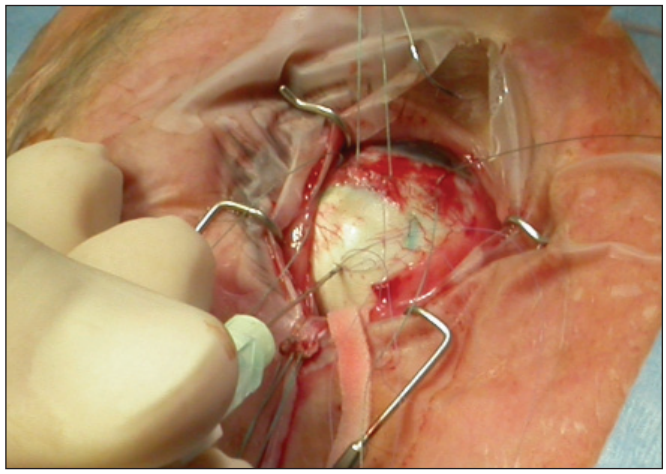

Fig. 1 - *Fine needle aspiration biopsy sample: transscleral approach in a posterior uveal melanoma.*

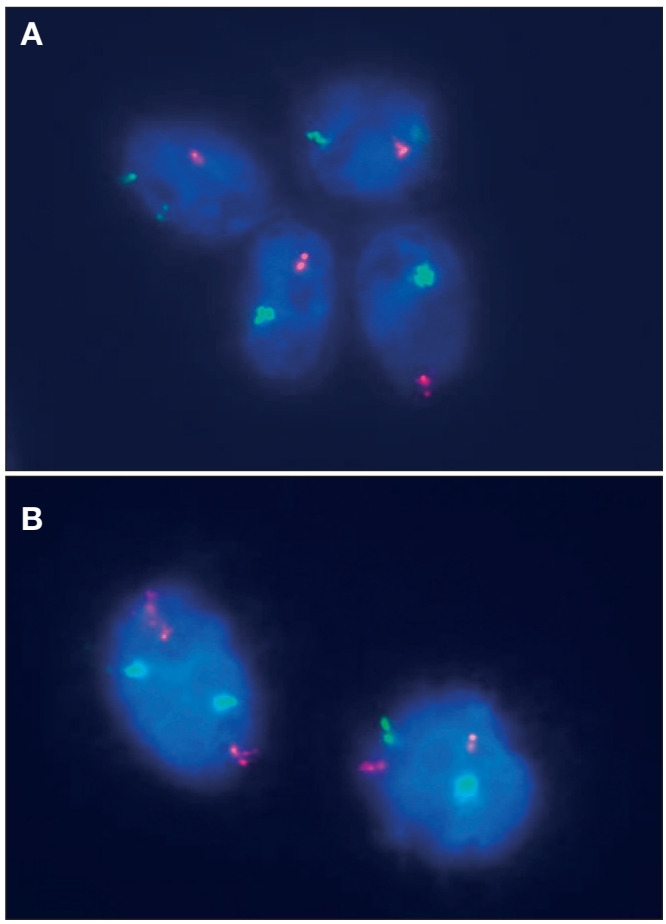

Fig. 2 - *Fluorescence in situ hybridization analysis of tumor material sampled by fine needle aspiration biopsy. (**A**) A case with monosomy 3: each cell has two chromosomes 10 (labeled in green as controls), but only one chromosome 3 (labeled in red). (**B**) A case with disomy 3: each cell has two chromosomes 3 (in red) and two chromosomes 10 (in green).*

NJ. USA) 1400 U/mL at 37 °C for 2 hours. Then the suspension was washed in RPMI 1640 and used to prepared cytospins. Slides were fixed with a cytologic fixative (Bio-Fix; Bio-Optica, Milano, Italy), and stored at –20 °C. FISH analysis was performed with a centromeric probe for chromosome 3 labeled with SpectrumOrange and centromeric probe for chromosome 10 labeled with SpectrumGreen (Abbott-Vysis, Downers Grove, IL, USA) following the manufacturer's procedure. Slide and probe were codenatured in Hybrite' (Vysis) at 75 °C for 5' and hybridized in a humid chamber overnight at 42 °C. Post-hybridization washes were made at 73 °C in 0.4 x SSC/0.3% NP-40 for 2' and at room temperature in 2 x SSC/0, 1% NP-40 for 1'. Slides were air dried and mounted with a Vectashield, mounting medium with DAPI (Vector Laboratories, Burlingame). Microscope analysis was carried out with a fluorescent microscope (Zeiss Axioplan fluorescent microscope, Germany) equipped with a cooled charge-coupled device (CCD) camera (Hamamatsu, Hamamatsu-city, Japan) and appropriate single band and triple band filters. Images were analyzed using CROMOFISH software (Amplimedical, Assago-MI, Italy). At least 100 cells were evaluated for each case; loss of chromosome 3 was reported when more than 15% of cells showed a single signal for chromosome 3 (Fig. 2).

RESULTS

We studied 8 patients (4 male and 4 female) with a mean age of 75 ± 8.75 years with medium-sized choroidal melanoma with ciliary body invasion. Demographic characteristics, tumor characteristics, and samplings results are showed in detail in Table I.

Mean tumor thickness was 8.2 ± 2.1 mm (range 5.5–10 mm) and largest basal diameter was 10.6 ± 2.6 mm. Transscleral FNAB yielded sufficient material in 7 of 8 eyes (87.5%). Monosomy 3 was detected in 5 (71.4%) cases and disomy 3 in the remaining 2 (28.6%) cases. Among tumors with monosomy 3, the percentage of monosomy 3 cells in every sample ranged from 73% to 96% (mean 89% ± 9.4). No complications were observed during the surgical procedure or during follow-up. No patients developed any local recurrence or metastatic disease.

DISCUSSION

Prognostication of metastatic disease in patients with posterior uveal melanoma is limited to eyes that undergo enucleation or tumor resection (10-16). Because conservative treatment by radiotherapy is the current standard in the treatment of choroidal melanoma, most of the patients treated for posterior uveal melanoma have no reliable information about their life prognosis. Moreover, clinicians are unable to accurately select patients at high risk for metastasis to be included in ongoing studies of adjuvant chemotherapy.

Recently monosomy 3 has been demonstrated to be a highly reliable parameter to select patients with uveal melanoma at high risk for metastasis (10-16). Monosomy 3 has been checked in enucleated eyes or in resected tumors, but no *in vivo* study has been previously performed to detect this genetic alteration in uveal melanomas (5).

FNAB is a cytologic sampling technique used for the di-

TABLE I - DEMOGRAPHIC AND CLINICAL DATA OF SAMPLED EYES

No.	Age, yr	Eye	Location	LBD, mm	Thickness, mm	Anterior margin	Posterior margin	FNAB sample	Monosomy 3	% of positive cells	MTD
1	82	OD	N	11	9	CB	Post E	Sufficient	No	0	No
2	81	OS	NS	7	10	CB	Pre E	Sufficient	No	0	No
3	73	OD	NS	12	7.5	CB	Post E	Sufficient	Yes	73	No
4	82	OD	TS	12	5.5	CB	Post E	Sufficient	Yes	94	No
5	82	OD	TS	11	6.7	CB	Post E	Sufficient	Yes	96	No
6	60	OS	NI	10	8.5	CB	E	Sufficient	Yes	92	No
7	75	OD	TI	15	10	CB	Post E	Sufficient	Yes	84	No
8	65	OD	N	7	8.5	CB	Pre E	Insufficient	___	___	No

LBD = Largest basal diameter; FNAB = Fine needle aspiration biopsy; MTD = Metastatic disease; OD = Right eye; N = Nasal; CB = Ciliary body; E = Equatorial; OS = Left eye; S = Superior; T =Temporal; I = Inferior

agnosis of uncertain intraocular tumors (17-20). This technique has already been used to sample enucleated eyes to investigate cytogenetic abnormalities with promising results (2, 8). To our knowledge, FNAB has never been previously used to analyze *in vivo* chromosomal abnormalities of intraocular tumors (5).

We consider that moving detection of monosomy 3 from ex vivo to *in vivo* may represent a relevant improvement in the prognosis of patients with uveal melanoma. Our data demonstrate that FNAB yielded adequate material to detect monosomy 3 in about 90% of sampled cases. When monosomy 3 is present most of the cells carry this genetic abnormality. No complications were detected during the follow-up.

In conclusion, this preliminary study demonstrates that medium-sized choroidal melanomas may be safely and adequately sampled to detect monosomy 3 using transscleral FNAB. We are currently investigating the application of this technique to small and large choroidal melanomas.

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