ARTICLE

Clinical applications of chromosome analysis, from fine needle aspiration biopsies, of posterior uveal melanomas

KAREN SISLEY, CARMEL NICHOLS, M. ANDREW PARSONS, ROBIN FARR, ROBERT C. REES, IAN G. RENNIE

Abstract

Purpose **An accurate assessment of prognosis is essential to the clinical assessment of malignancy. In posterior uveal melanoma specific chromosome alterations have been shown to correlate significantly with prognosis; but the procedure is restricted to patients treated surgically, and in consequence has been limited mainly to large tumours. Fine needle aspiration biopsy (FNAB) may provide sufficient material to perform this technique, and allow its use in the *in situ* assessment of tumours, including small lesions. To determine the feasibility of this approach we have conducted a pilot study using enucleated tumours.**

Methods **Ten cases of posterior uveal melanoma were studied. In each instance both a test FNAB and a standard tissue preparation were conducted, and the results compared. FNABs were obtained from enucleated tumours by aspirating cells using a 5 ml syringe with a .25 gauge needle; cells were injected into phosphate-buffered saline, spun down and established *in vitro*. Conventional short-term cultures were established from tumour tissue samples, which were minced prior to the establishment of cultures. Cytogenetic analysis was performed following standard protocols.**

Results **Of the 10 cases examined, full chromosome analysis was obtainable from all standard tissue short-term cultures. Cytogenetics was successful from cultures of 6 FNAB, with 2 further FNAB producing partial analyses. No major clonal differences were determined between the two procedures.**

Conclusions **Cytogenetic analysis of FNAB appears to be entirely feasible for posterior uveal melanomas, and may permit an accurate *in situ* assessment of tumours, including small lesions.**

Key words Uveal melanoma, Cytogenetics, Prognosis *in situ*

The cytogenetic analysis of leukaemias has relevance for both the diagnosis and the prognosis of this malignancy,[1] but for most solid tumours chromosome analysis is highly problematic and cytogenetic information is limited with little, or no, clinical benefit.[2-4] Posterior uveal melanomas are unusual in this respect, as they appear to be relatively amenable to cytogenetic analysis, approximately 100 cases having been reported to date.[5-12] Furthermore consistent patterns of chromosomal involvement have been observed, with chromosomes 1, 3, 6, 8 and Y most frequently implicated.[5-12] Increasing evidence suggests that certain chromosome abnormalities are more common to tumours of a specific location; in particular tumours with a ciliary body component almost invariably possess alterations of chromosomes 3 and 8 in association.[6-12] Likewise, choroid melanomas appear to have higher levels of involvement for chromosome 6 and 11,[6,7,12] whilst iris melanomas seemingly exhibit a different repertoire of alterations.[13]

Recently, the significance of chromosome changes in posterior uveal melanoma has been clearly demonstrated, with certain chromosome abnormalities, loss of chromosome 3 and additional 8q, having been shown to be a significant predictor not only of survival, but also of disease-free interval.[14,15] Furthermore, as a prognostic indicator, initial evidence suggests that cytogenetic analysis may be more reliable than other classical prognostic indicators.[14,15] In uveal melanoma, the clinical value of cytogenetic analysis seem apparent; however, the procedure requires fresh tumour material, and as a necessity is therefore restricted to surgically treated tumours. In practice analysis is biased towards large tumours that cannot

K. Sisley ✉
Institute for Cancer Studies
Medical School
Beech Hill Road
Sheffield University
Sheffield S10 2RX, UK
Tel: +44 (0)114 271 2237
Fax: +44 (0)114 271 3515

K. Sisley
C. Nichols
R. Farr
I.G. Rennie
Department of Ophthalmology and Orthoptics
Sheffield University
Sheffield
UK

M.A. Parsons
Ophthalmic Sciences Unit
Sheffield University
Sheffield
UK

R.C. Rees
Department of Life Sciences
The Nottingham Trent University
Nottingham
UK

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Table 1. *Clinicopathological details of the patients*

Case no.	Age (years)	Sex	Tumour location	Cell type	Mean tumour diameter (mm)
1	80	M	CB/C	Mixed	19.65
2	69	F	C	Mixed	19.1
3	70	F	C	Spindle	15.45
4	67	F	CB	Mixed	16.2
5	78	F	CB	Mixed	15.15
6	76	F	C/CB	Spindle	12
7	56	M	CB	Mixed	16.7
8	58	M	CB	Mixed	15.2
9	82	F	CB/C	Mixed	18.25
10	58	M	C/CB	Mixed	18.05

reasonably be treated by more conservative means. Chronologically, changes of chromosome 3 appear to occur earlier than other chromosome alterations,[15, 17] but it is unknown what abnormalities small tumours exhibit, or whether cytogenetic analysis would be of benefit in the assessment of these potentially early lesions. To explore the possible application of cytogenetics to the assessment of small lesions, we have conducted a feasibility study, using test fine needle aspiration biopsy (FNAB), on surgically removed tumours.

Materials and methods

Ethics approval and informed patient consent were obtained for the removal of tissue prior to commencing the study. A total of 10 tumours was examined, and the clinicopathological data of the patients are presented in Table 1.

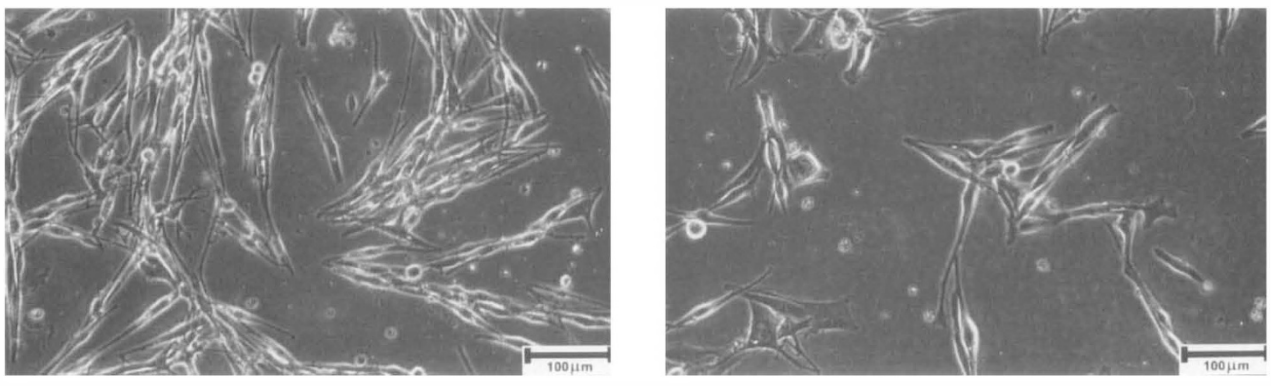
For each tumour both a conventional procedure and a test FNAB were conducted. FNAB were performed on the enucleated tumours, by aspirating cells using a 5 ml syringe with a .25 gauge needle, directly from the tumour mass once the globe had been opened. Tumour cells were injected into phosphate-buffered saline (PBS), spun down, and established as cultures. Tumour tissue for conventional short-term cultures was transported to the laboratory in PBS. Upon receipt the specimen was processed by fine mincing with scalpel blades, washing in PBS and resuspending in medium. Medium for both techniques was RPMI supplemented with 20% fetal calf serum, glucose (2 mg/ml), fungizone (3 mg/ml), penicillin (100 U/ml), streptomycin (100 mg/ml) and epidermal growth factor (10 ng/ml). For both procedures tumour cells were seeded into flat-bottomed tissue culture test-tubes and grown in culture, at 37 °C with 5% CO_2.

Cytogenetic analysis was performed using standard techniques,[6] with an Olympus microscope and the Powergene system (Perceptive Scientific International) and abnormalities reported in accordance with the ISCN.[18]

Results

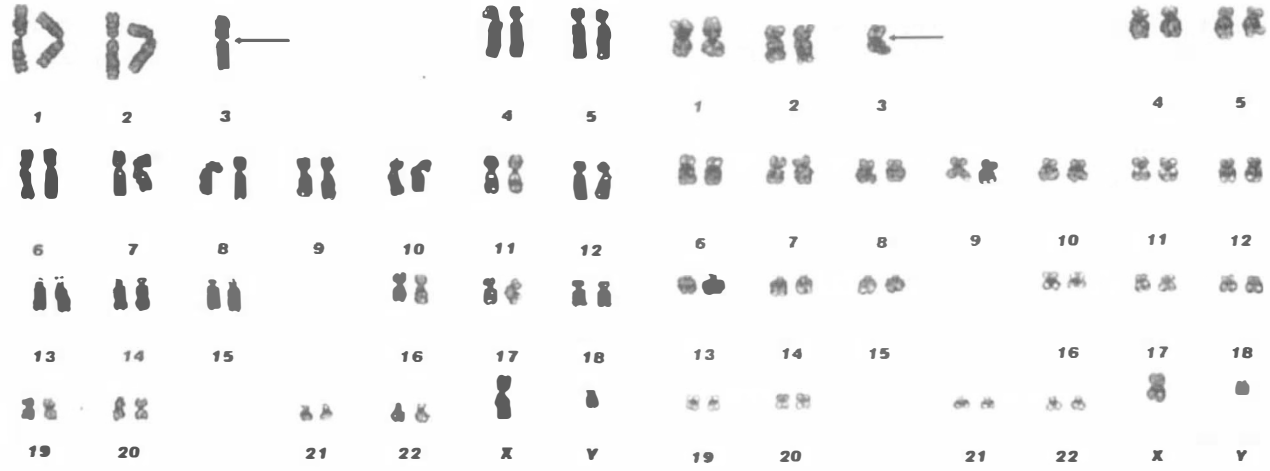
Both conventional short-term cultures and those established from FNAB were harvested at the same time, at 7 days after initiation of cultures. Growth, in all instances, was more substantial in the conventional short-term cultures (Fig. 1) and, as expected, when the conventional cultures were harvested they produced more material than the FNAB cultures. For comparative purposes the same number of slides was prepared from all samples. The results of cytogenetic analysis, for both procedures, are recorded in Table 2; only the clonal changes are reported. Representative karyotypes from both the short-term and FNAB analysis of one tumour (case 10) are shown in Fig. 2.

The series was composed of a representative admixture of tumour locations and cell types, and no bias for producing successful cytogenetic analysis was found. Of the 10 tumours analysed, results were obtainable from all the conventional short-term cultures and analysis was



(a) (b)

Fig. 1. *Comparison of growth in culture, from the same uveal melanoma, at 7 days from a standard tissue culture (a) and a FNAB culture (b). Substantially more growth was found with conventional procedures compared with FNAB cultures.*



(a) (b)

Fig. 2. *Representative karyotypes from the standard tissue preparation (a) and the FNAB culture (b) of case 10. The same clonal alterations were found in both preparations, and are indicated by arrows.*

successful from 6 of the test FNAB. A further 2 FNAB samples produced incomplete analysis (cases 5 and 8), which, with the examination of the remaining cell harvest, would have produced full analyses, as confirmed subsequently (data not shown). The results of 2 FNAB were considered complete failures (cases 6 and 9).

Where analysis was possible on both procedures, comparison of the results demonstrated that all major clonal chromosome changes were present in both the conventional culture and its corresponding FNAB sample. In most instances, the less frequent clonal alterations were also identified by both procedures, although on occasions (cases 2, 5 and 7) the lower

Table 2. *Comparison of cytogenetic analysis from conventional short-term cultures, and fine needle aspiration biopsy cultures*

Case no.	Conventional short-term cultures	Fine needle aspiration biopsy cultures
1	11 cells 44–46,X,−Y[11],der(1)t(1;8)(q10;q10)[11],−3[11],+10 [3],del(11)(q?)[3],+21[6],+22[2][cp11]	10 cells 44,X,−Y,der(1)t(1;8)(q10;q10),−3 2 cells 45,X,−Y,der(1)t(1;8)(q10;q10),−3,+21 3 cells 44,X,−Y,der(1)t(1;8)(q10;q10),−3,del(11)(q?)
2	3 cells 48,X,−X,del(1)(p36),+del(1)(p36),−3,i(8) (q10),+i(8)(q10)x2,+der(8)t(8;8)(p23;q23)	3 cells 48,X,−X,del(1)(p36),+del(1)(p36),−3,i(8)(q10),+i(8) (q10)x2,+der(8)t(8;8)(p23;q23) 4 cells 48,X,−X,del(1)(p36),+del(1)(p36),−3,i(8)(q10),+i(8) (q10)x2,+der(8)t(8;8)(p23;q23),i(17)(q10)
3	13 cells 46,XX,der(6)t(6;8)(q16;q13),der(11)t(6;11) (p21;q23),add(11)(p15),der(20)t(6;20)(p11;p11)	7 cells 46,XX,der(6)t(6;8)(q16;q13),der(11)t(6;11) (p21;q23),add(11)(p15),der(20)t(6,20)(p11;p11)
4	2 cells 44,X,−X,−1,−3,i(8)(q10),der(8)t(8;8)(p22;q21), del17(q21q22),der(19)t(9;19)(q13;p13),der(22) t(1;22)(q11;p11),+mar 11 cells 45,X,−X,−1−3,i(8)(q10),der(8)t(8;8)(p22;q21), del17(q21q22),der(19)t(9;19)(q13;p13),+21,der(22) t(1;22)(q11;p11),+mar 2 cells 44,X,−X,−1−3,i(8)(q10),der(8)t(8;8)(p22;q21), −10,del17(q21q22),der(19)t(9;19)(q13;p13),+21, der(22)t(1;22)(q11;p11),+mar	5 cells 45,X,−X,−1−3,i(8)(q10),der(8)t(8;8)(p22;q21), del17(q21q22),der(19)t(9;19)(q13;p13),+21,der(22) t(1;22)(q11;p11),+mar 2 cells 44,X,−X,−1−3,i(8)(q10),der(8)t(8;8)(p22;q21),−10, del17(q21q22),der(19)t(9;19)(q13;p13),+21,der(22) t(1;22)(q11;p11),+mar
5	3 cells 46,XX,−3+i(8)(q10)	5 cells 35−44,XX,−3[5],−4[3],add(6)(q11)[2],i(8)(q10)[5], +i(8)(q10)[5],+2mar,[cp5],inc
6	10 cells 44−47,XX,del(2)(q?)[10],−5[3],−6[3],+add(6)(q12) [6],−7[4],add(8)(q24)[4],−10[4],+11[3],add(12)(p13) [10],−14[6],+19[3],−21[3],+22[2],+3mar[6][cp10]	Failed
7	8 cells 39−43,X−Y[8],−1[4],−2[3],−3[3],i(8)(q10)[8], +i(8)(q10)[8],−15[3],−17[3],−20[3],+22[2]	5 cells 41−45,X−Y[5],−3[5],i(8)(q10)[5],+i(8)(q10)[5],−20[3] +22[2][cp5]
8	12 cells 45−47,X−Y[3],−7[3],+22[8],+2mar[4][cp12]	3 cells Analysis incomplete; failed
9	7 cells 46,XX	Failed
10	5 cells 45,XY,−3 5 cells 45,XY,−3,i(8)(q10),del(18)(p11) 2 cells 45,XY,−3,i(8)(q10),del(18)(p11),del(20)(p11p11)	3 cells 45,XY,−3 6 cells 45,XY,−3,i(8)(q10),del(18)(p11) 3 cells 45,XY,−3,i(8)(q10),del(18)(p11),del(20)(p11p11)

number of cells available prevented these alterations being considered clonal. Additional anlaysis of the remaining cells harvested subsequently confirmed the clonal nature of these abnormalities (data not shown).

Discussion

Increasingly, new methods have been adapted and applied to the pathological assessment of FNAB, in a variety of malignancies, including flow cytometric assessment and mutational analysis.[19,20] Reports of cytogenetic analyses performed on FNAB from solid tumours are virtually non-existent and have achieved variable degrees of success.[21,22] In uveal melanoma cytopathological examination of FNAB has proved of value in the determination of prognosis,[23] and this approach, combined with assessment of prognostically relevant chromosome alterations,[14,15] may prove highly appropriate.

Uveal melanomas have previously been determined as suitable for cytogenetic analysis,[5-15] and this investigation implies that the technique is applicable to both short-term and FNAB cultures of all uveal melanomas independent of tumour location or cell type. *In vitro* growth was established from all the conventional short-term cultures and, although substantially less, also from all the corresponding FNAB cultures. Karyotypes were obtainable from all standard short-term cultures, whilst 60% of the FNAB cultures provided adequate numbers of cells for assessment. In reality the success rate for FNAB may be higher (80%), as continued examination of the remaining material from the partial analyses (cases 5 and 8) provided sufficient cells for completion.

Previous attempts to utilise cytogenetic assessment of FNAB from tumours have indicated differences between the genetic alterations identified by the two methods.[22] In this investigation, all major clonal alterations were detectable by both procedures. In several instances (cases 2, 4, 5 and 7), the small number of cells examined prevented the original identification of all clonal aberrations, but these were latterly confirmed by extended analysis. It is also of interest that changes which would normally be considered as non-clonal, such as loss of chromosome 10 in case 4, were represented at the same low level in both short-term and FNAB techniques. The results of this investigation suggest that analysis of the FNAB cultures provides an accurate reflection of the cytogenetic alterations of the tumour, despite the sampling of small areas, and may imply a greater tendency for homogeneity of genetic alterations amongst uveal melanomas. The general agreement in uniformity of results from cytogenetic studies of these tumours,[5–15] identifies a clear consistency in their chromosome alterations, which this investigation implies can be extended to the sampling of nominal regions of the melonoma.

The recent evidence suggesting that chromosome alterations are an excellent predictor of prognosis in uveal melanoma,[14,15] combined with the results of this study, suggesting the suitability of these melanomas for FNAB cytogenetic analysis, indicates that this procedure can, in theory, be reasonably performed on small lesions prior to surgical intervention. Although the procedure in principle seems entirely suited to the assessment of lesions *in situ*, on a cautionary note it is important to consider that these were test FNAB and performed by direct puncture of the tumour under ideal circumstances. However, success rates *in situ* could be bolstered by using fluorescent *in situ* hybridisation (FISH) of probes for the prognostically significant chromosome changes. This report indicates that cytogenetic analysis of FNAB cultures from uveal melanomas is feasible, yielding representative chromosome analysis, and would suggest a real application for the procedure in the assessment of these tumours.

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