Detection of Genetic Prognostic Markers in Uveal Melanoma Biopsies Using Fluorescence *in Situ* Hybridization

Nicole C. Naus, Anette C. A. Verhoeven, Ellen van Drunen, Rosalyn Slater, Cornelia M. Mooy, Dion A. Paridaens, Grégorius P. M. Luyten, and Annelies de Klein[1]

Departments of Ophthalmology [N. C. N., A. C. A. V., G. P. M. L.], Cell Biology and Genetics [N. C. N., E. v. D., R. S.], Clinical Genetics [R. S., A. d. K.], and Ophthalmopathology [C. M. M.], Erasmus University Rotterdam, and The Rotterdam Eye Hospital [D. A. P.], 3000 DR Rotterdam, the Netherlands

ABSTRACT

Purpose: **In uveal melanoma, specific chromosomal abnormalities are known to correlate with the risk of metastases; changes in chromosomes 3 and 8q correlate strongly with a decreased survival of the patient, whereas chromosome 6 abnormalities are associated with a better prognosis. Usually, karyotyping and fluorescence *in situ* hybridization (FISH) analysis are used to detect these abnormalities in resected tumor tissues. However, the evaluation of these chromosomal changes is compromised in patients treated with eye-retaining treatment protocols because of the lack of tumor material. The purpose of this study was to validate the use of FISH for the analysis of genetic prognostic markers.**

Experimental design: **We analyzed 40 uveal melanoma fine needle aspiration biopsies (FNABs) and the corresponding main tumor with FISH.**

Results: **All biopsies were found to contain tumor cells, and FISH analyses of the samples were successful in all cases. Statistical analysis showed very good agreement between the FISH results from the biopsies and those from the main tumor. In only 2 of 249 hybridizations did we find a small variation that could have led to a misclassification.**

Conclusions: **Our results indicate that the application of FISH to FNABs is a reliable method for assaying genetic prognostic parameters such as chromosome 3 loss and/or chromosome 8q gain. Implementation of this method in a diagnostic setting means that we are able to identify patients at risk of developing metastatic disease, not only in enucleated patients but also in cases treated with conservative treatment modalities such as radiotherapy.**

Received 8/14/01; revised 10/22/01; accepted 11/14/01.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Department of Clinical Genetics, Erasmus University Rotterdam, P. O. Box 1738, 3000 DR Rotterdam, the Netherlands. Phone: 31-10-408-7227; Fax: 31-10-4087200; E-mail: deklein@kgen.fgg.eur.nl.

INTRODUCTION

Uveal melanoma is the most common primary intraocular tumor with an annual incidence of 0.7/100,000 in the Western population (1). Although <2% of the patients have clinically detectable metastases at presentation, ~50% of all patients ultimately die of metastatic disease. The median survival after the diagnosis of metastasis is extremely poor. No effective treatment exists for metastatic disease, but new approaches for the management of metastases, involving IFN, interleukin, and a combined chemo- and immunotherapy are under study (2, 3).

The predictive value of classic histological prognostic parameters such as tumor size, vascular patterns, and histological cell type has been analyzed in several retrospective studies. Additional clinical parameters associated with a poorer prognosis are extrascleral growth, tumor location (ciliary body), older age, and male gender (4). Prescher *et al.* (5) showed that loss of chromosome 3, in comparison with tumor location or tumor diameter, is a better prognostic parameter of relapse-free and overall survival. Sisley *et al.* (6) confirmed the prognostic value of monosomy 3 and, in addition, demonstrated a strong inverse correlation between the presence of additional copies of 8q and survival. In contrast, patients with tumors having chromosome 6 abnormalities appear to have a better prognosis (7).

Although enucleation is still indicated in large uveal melanoma, radiotherapy (such as proton beam irradiation or plaque therapy, with or without transpupillary thermotherapy) has become the first choice of treatment for patients with small- or medium-sized melanomas (8). A clear advantage for the patient is saving the eye and vision, but the lack of histological specimens compromises the evaluation of the prognostic markers. Because FNABs have been used successfully for several years for intraocular tumor diagnosis (9, 10), the analysis of FNABs[2] offers an attractive and safe alternative in these cases.

In the present study, we have investigated the possibility of applying FISH analysis to FNABs. FNABs were obtained *ex vivo*, *i.e.*, after enucleation of the tumor-containing eye, and directly used for FISH analyses. To investigate whether these FNAB samples are a good representation of the main tumor, larger samples of the main tumor were processed for direct FISH analysis and conventional cytogenetics. The results of the FNAB were compared with those of the main tumor.

MATERIALS AND METHODS

Patients and Tumor Samples. From January 1997 to December 1999, we collected FNABs and matched tumor material from 40 uveal melanoma patients. These patients were referred for enucleation of the affected eye to the Department of

[2] The abbreviations used are: FNAB, fine needle aspiration biopsy; FISH, fluorescence *in situ* hybridization; YAC, yeast artificial chromosome.

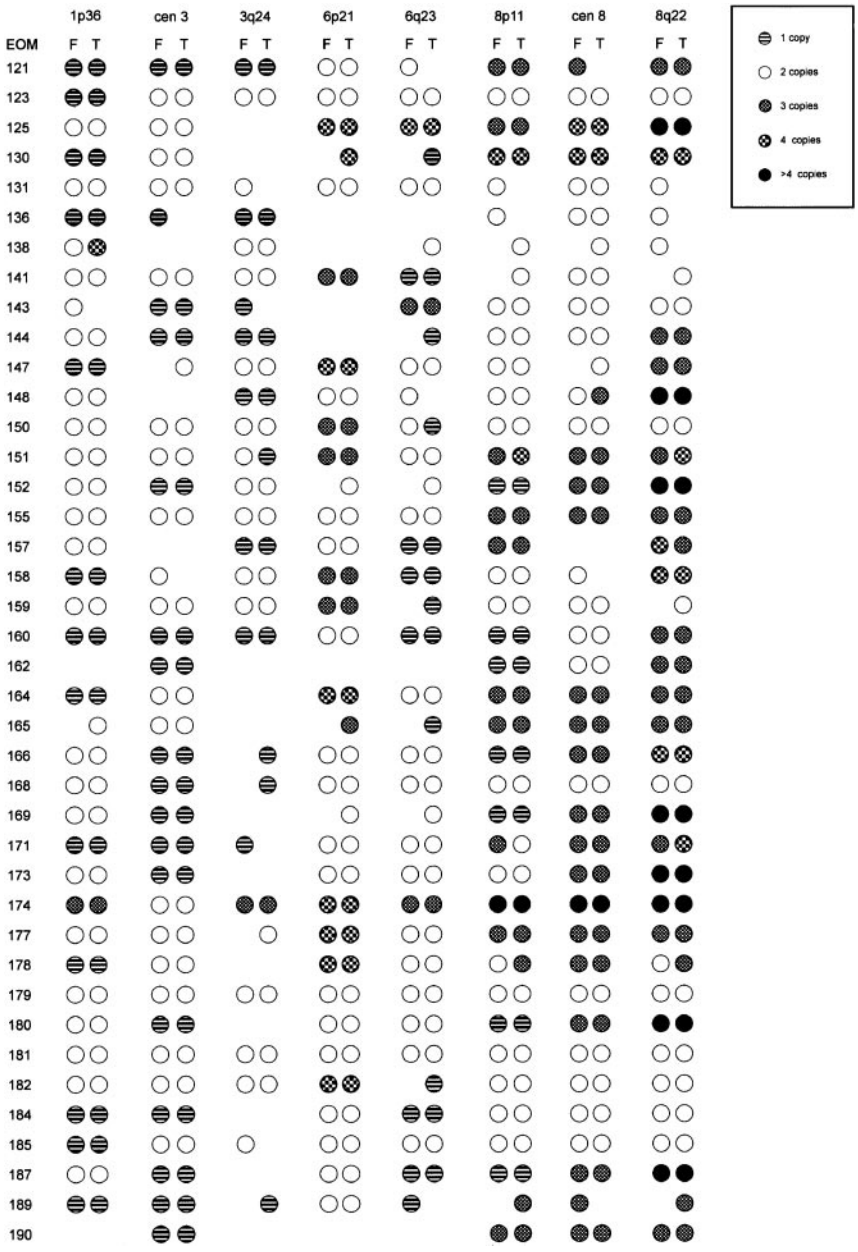

Fig. 1 Results of the FISH studies carried out on 40 FNABs of uveal melanoma and the corresponding tumor.

Ophthalmology, University Hospital Rotterdam and the Rotterdam Eye Hospital. Informed consent was given before enucleation, and the study was performed according to the tenets of the Declaration of Helsinki. The mean age was 60.5 years (range, 34–85); 19 patients were male, and 21 were female. The mean tumor diameter was 12.9 mm (range, 7–18 mm). Five tumors were derived from the ciliary body and 35 from the choroid. Immediately after enucleation, transvitreal FNABs were taken, and both the FNABs and fresh tumor material were processed for FISH and/or cytogenetic analysis according to standard procedures. Histopathological diagnosis and treatment of the patient were not compromised by biopsy collection. Cytogenetic studies were carried out on peripheral blood samples of each of the patients to exclude the presence of congenital balanced chromosome abnormalities.

FNABs. After enucleation, the bulbus was first transilluminated to define the location of the tumor. A 25-gauge needle attached to a 10-ml syringe was inserted through the sclera into the tumor, and suction was applied to the syringe. After sampling, the pressure was equalized before removal of the needle to avoid seeding of tumor cells. Cells were collected in culture medium, fixed in methanol:acetic acid, and FISH preparations were made as described (11).

Fresh Tumor Material. Tumor specimens, collected after *ex vivo* FNABs, were processed as described (12). For direct FISH, 1 ml of the cell suspension was fixed, and the remaining

Table 1 Cytogenetic analysis of the main tumor

Tumor	Karyotype of main tumor (ISCN, 1995)
EOM-121	46-47,XY,del(1)(p31p36),−3,der(4)t(1;4)(q12;q21),+8,+21[cp3]/45,X,−Y[3]/46,XY[8]
EOM-123	46,XY[22]
EOM-125	72-76,XXX,dic(1;7)(p10;p14),+dic(1;7)(p10;p14),−3,+4,+6,−7,+i(8)(q10),+9,−11,−15,+16, +18,+20,+?21,+22[cp12]/49-54,idem[cp2]
EOM-130	41-48,XX,der(1)t(1;6)(p11;p?2),add(4)(q1?2),−5,−6,+7,+8,+8,−9,add(11)(q13-14), add(11)(q13-14),−13,−16,+22,+mar.ish der(6)(wcp6+),+mar.ish der(16)t(6;16) (wcp6+,wcp16+)[cp6]
EOM-131	46,XX[11]
EOM-136	41-44,XX,der(1;8)(q10;q10).ish;der(1;8)(wcp8+,2053b3+,p1.164+,D8Z2+,puc1.77+,wcp1+), −3,+der(8).ish;der(8)ins(p?21q?23q?24.1)del(8)(q22q22)(wcp8+,114C11+,105H8+, p1.164+,2053b3+),−15,del(16)(q11q1?3).ish;del(16)(wcp16+,pHUR195−)[cp15]
EOM-141	46,XY,+2,dic(6;13)(q12;p10),dic(6;14)(q12;p10)[16]
EOM-147	44-47,XY,del(1)(p2?)[5],add(7)(p?2)[4],+9[2],−15[4],add(19)(q1?3)[3],+mar[2][cp6]/46,XY[3]
EOM-148	47,XY,+?der(2)[2]/46,XY,add(8)(q10),der(15)t(1;15)(q11;p11)[1]/45,X,−Y[3]/46,XY[10]
EOM-150	47,XY,+9,der(10)t(6;10)(p12;q26)[4]/47,XY,+9[3]/46,XY[4]
EOM-151	46,XX,der(20)t(6;20)(p12;p12)[5]/47,idem,+8[4]/47,idem,+8,psudic(17;15)(p13;p11)[3]/46,XX[3]
EOM-152	45-48,XX,−3,i(8)(q10),+i(8)(q10),+i(8)(q10)[cp5]/47-49,XX,+3[3],+6[2],+6[2][cp4]/46,XX[4]
EOM-157	47,XX,+8[7]/46,XX[10]
EOM-158	45,X,−Y[11]/46,XY[4]
EOM-159[a]	40-46,XX,der(2)t(2;6)(q3?4;?),der(5)t(5;6)(q34;?),del(6)(q?)[3], der(7)t(7;11)(p21;?)t(8;11)(q?; ?),der(10)t(8;10)(?;p1?4),der(11)t(8;11)(?;q1?4),der(16)t(8;16)(q?;q24)[7],der(18)t(17;18)(?; q23)[cp19]/46,XX[1]
EOM-160	40-42,XX,del(1)(p21),−3,−6,i(8)(q10)[1],−12,−18[cp5]/47,XX,del(1)(q?),der(1)t(1;8)(p?;q?),+7, −8,+9,del(11p)[1]/46,XX[10]
EOM-165[a]	40-46,XY,der(6)t(6;6)(q16;?),der(7)t(1;7)(q12;q36),+8[cp20]
EOM-166	47,X,−X,−3,+7,i(8)(q10)[1]/idem tetraploid[1]/46,XX[4]
EOM-174	75,XXY,add(1)(p),−3,+i(6)(p),i(8)(q),add(9)(p)[1]
EOM-177	45-47,XY,−5[2],+8[2],add(8)(p22),+add(8)(p22)[4],−9[2],del(13)(q?14q?21),der(17)ins(17; 13)(q12;?q14q21)del(17)(q22q23),−19[2],−22,+mar[cp7]
EOM-178[a]	45-47,XX,der(1)t(1;15)(p11;?),der(5)t(5;14)(p?;?),der(7)t(6;7)(?;q36),+8[2],−14[5],−15, der(17)t(7;17)(?;p12)t(6;7)(?;?),+ r(5)[cp10]
EOM-179	46,XY[15]
EOM-180	43-45,X,−X,−3,i(8)(q10),+i(8)(q10),+i(8)(q10)[2],der(16;21)(q10;q10),der(22)[1][cp5]/ tetraploid,idem,inc[2]
EOM-182[a]	46,XX,der(6)t(6;6)(q16;p12), der(22)t(8;22)(?;p11), der(22)t(6;22)(?;q13)[16]
EOM-187	45,X,−Y,−3,−4,i(8)(q10),+i(8)(q10),+mar[1]
EOM-189	44,XY,−1,−2,−3,dic(1;6)(q10;q10),+ring[5]/45,idem,+8,[12]/90,XXYY,idem, +8, +8[1]

[a] In these cases, conventional cytogenetics were supplemented with spectral karyotyping (15).

cell suspension of the tumor was cultured. For cytogenetic analysis, cells were incubated with Colcemid (0.15 μg/ml) for 6 h at 37°C and fixed. The chromosome preparations were stained with acridine orange or atebrine to obtain R or Q banding. Cytogenetic abnormalities were described in accordance with the International System for Human Cytogenetic Nomenclature (Basel, 1995). Remaining cultures were stored in N_2 and in one instance (EOM-121) used for additional FISH studies. FISH analysis on metaphases using locus-specific probes, and whole chromosome painting was carried out to further characterize the abnormalities in cases where cytogenetic analyses revealed the presence of partially defined chromosomes.

FISH. Dual color FISH was performed using centromeric and locus-specific cosmid, P1, or YAC probes for chromosomes 3 and 8. Probes for chromosomes 1 and 6 were used if sufficient material was available. FISH was performed using standard procedures (11). The concentration for centromeric probes was 5 ng/slide; for cosmids, P1, and YAC probes, 50–75 ng/slide were used. The probes used were p1–79 (1p36), Pα 3.5 (centromere 3), YAC 827D3 (3q24), cos85 (6p21), and cos52 (6q23) (Prof. Y. Nakamura, Tokyo, Japan), Cos105H8 (8p11), D8Z2 (centromere 8), and ETO (8q22). After hybridization and washing, slides were counterstained with 4′,6-diamidino-2-phenylindole and mounted in anti-fade solution (Dabco-Vectashield 1:1). Signals were counted in 300 interphase nuclei according to the criteria of Hopman *et al.* (13). Cutoff limits for chromosome loss (15% of the nuclei with one signal) or gain (>10% of the nuclei with three or more signals) were adapted from the available literature (14). Variation found by FISH (in FNAB and the main tumor) were subdivided into five categories (Fig. 1): loss of one copy, normal copy numbers (two copies), gain of one copy, gain of two copies, and gain of more than two copies. When different subclones were identified, only the FISH findings of the largest clone were used to classify the material.

Statistical Analysis. To evaluate the agreement between the FISH results obtained from the FNAB and those obtained from the direct preparations of the tumor, we calculated the overall kappa, as well as the kappa of all probes separately. Kappa <0.20 was considered as a poor strength of agreement, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as good, and 0.81–1.00 as very good. The Wilcoxon's matched pairs signed rank sum test was used to compare the number of FISH

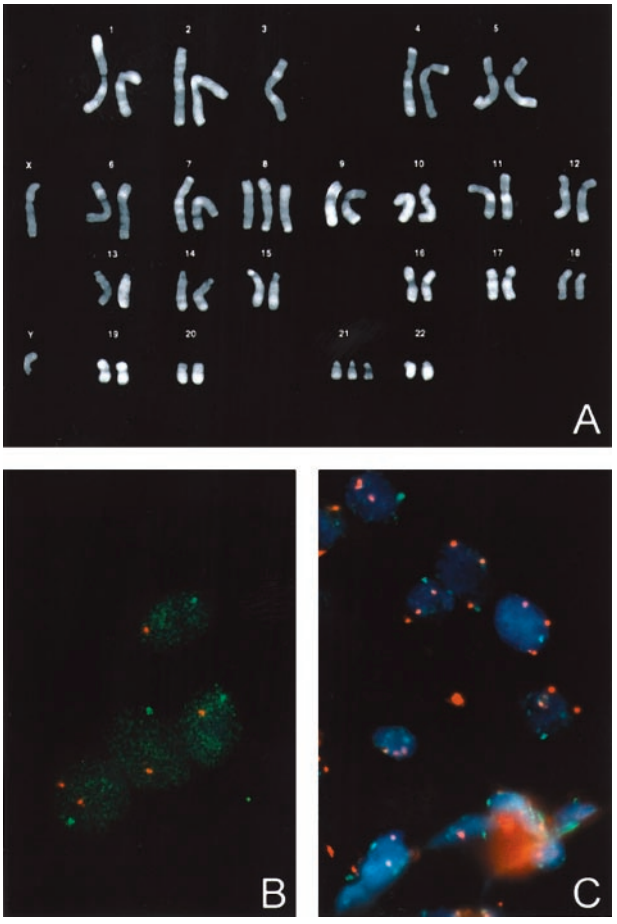

Fig. 2 *A*, karyotype of patient EOM-121: 47 XY,del(1)(p31p36),−3, der(4)t(1;4)(q12;q21),+8,+21. *B*, nuclei isolated from short-term culture of the tumor specimen hybridized with chromosome 3q24 (*green*) and 1p36 (*red*) probes. *C*, nuclei isolated from FNABs hybridized with probes for centromere 3 (*green*) and centromere 8 (*red*).

probes successfully used on the FNAB samples with the number of probes used on the direct preparation.

RESULTS

Fig. 1 shows the results of the FISH analyses performed on 40 FNAB specimens taken from enucleated eyes of uveal melanoma patients and the corresponding primary tumors. The findings of the conventional karyotype studies on the primary tumors are given in Table 1. Examples of karyotype analysis and FISH of patient EOM-121 are shown in Fig. 2. In this case, no direct preparations could be made because of shortage of material, and a short-term culture (passage 1) was used for FISH and karyotype analysis of the main tumor.

FISH Results on the FNABs *versus* Direct Preparations of the Main Tumor. All FNABs yielded sufficient tumor cells to allow FISH analysis. The number of probes used in the FISH analysis on the FNABs was not significantly different from the number of probes used on the direct tumor preparations (Wilcoxon's matched pairs signed rank sum test, $P = 0.197$). In eight instances, all eight probes could be tested on the FNAB and the main tumor. In 39 of 40 FNABs, the FISH of both chromosomes 3 and 8 could be analyzed. In one case (EOM-138), the FNAB was of low quality, and as a result, only chromosome regions 1p36 and 3q24 could be analyzed.

In 11 of 249 hybridizations, discrepancies between the FISH results of the FNAB and those of the corresponding tumor were detected (summarized in Table 2). In three instances [regions 8p11/8q22 (EOM-151), 8q22 (EOM-157)], this variation was because of classification of the largest clone only (three *versus* four copies of chromosome 8). In one tumor (EOM-171), FISH results of the FNAB suggested an additional chromosome 8 (gain of all chromosome 8 probes), whereas the tumor suggested the formation of an isochromosome 8q (normal chromosome 8p11, gain of centromere 8, and gain of two copies of chromosome 8q22). Thus, in both cases, gain of chromosome 8q was observed. In 6 of 249 hybridizations, FISH showed normal copy numbers in the FNAB but abnormal numbers in the tumor samples. In five of these six hybridizations [EOM-148 (centromere 8), EOM-150 (6q23), EOM-151 (3q24), and EOM-178 (8p11/8q22)], this concerned small abnormal subclones (12–17% of nuclei) that were detected in the main tumor, whereas they remained undetected in the FNAB. In EOM-138, a subclone of 32% of nuclei showing gain of chromosome region 1p36 in the main tumor was not found when analyzing the FNAB. To investigate the agreement between the FISH results of the FNAB and those of the direct tumor preparation, we calculated the weighted kappa of all probes separately and an overall weighted kappa. The overall weighted kappa was 0.95 (range for the probes separately, 0.90–1.00), indicating a very good agreement between the FISH results obtained from FNABs and those obtained from the main tumor.

FISH Results *versus* Cytogenetic Analysis of Main Tumor. Cytogenetic analysis of the melanomas could be performed in 26 of 40 cases. We found variation between the FISH findings of the FNABs and main tumors and those expected from the cytogenetic analysis. In most instances (EOM-130, EOM-147, EOM-159, EOM-165, EOM-177, EOM-178, and EOM-182), the variation was attributable to the presence of partially defined or complex cytogenetic abnormalities and could be resolved by applying metaphase FISH using whole chromosome paints or spectral karyotyping (15). In other cases (EOM-123, EOM-157, and EOM-158), normal karyotypes were found after culturing, whereas FISH revealed abnormalities. In two cases (EOM-174 and EOM-187), only one metaphase could be analyzed because of poor tumor growth *in vitro*, and in 5 other cases (EOM-148, EOM-150, EOM-151, EOM-152, and EOM-160), minor variations were found between the cytogenetic and FISH results. In EOM-125, the FISH analyses revealed two copies for the chromosome 1p36 and centromere 3 probes. However, cytogenetic studies showed a triploid karyotype with only two copies of chromosome region 1p36 and disomy for chromosome 3. This indicates a relative loss of these chromosome regions in this tumor, although the FISH had shown no abnormalities.

DISCUSSION

In this study, we describe the application of FISH for determining of the presence of genetic abnormalities in FNABs

Table 2 FISH results of cases with discrepancies between FNAB and tumor[a]

Tumor	Locus	Material[b]	Copy number detected by FISH (% in FNAB/ tumor)[c]				
			1	2	3	4	>4
EOM-138	1p36	F/T	2/0	88/61	1/6	8/32	1/1
EOM-148	cen8	F/T	0/2	90/83	2/12	8/3	0/0
EOM-150	6q23	F/T	4/17	91/77	3/6	2/0	0/0
EOM-151	3q24	F/T	5/16	79/83	10/1	6/0	0/0
	8p11	F/T	0/0	22/8	41/15	36/77	1/0
	8q22	F/T	0/0	23/6	35/10	29/65	13/19
EOM-157	8q22	F/T	0/0	59/60	15/25	26/15	0/0
EOM-171	8p11	F/T	0/0	8/96	91/3	1/1	0/0
	8q22	F/T	0/0	7/2	89/13	4/84	0/1
EOM-178	8p11	F/T	2/0	98/87	0/12	0/1	0/0
	8q22	F/T	0/0	96/83	4/15	0/2	0/0

[a] Only cases in which differences in chromosome copy numbers were observed between FNAB and corresponding tumor are indicated in this table.

[b] F, FNAB; T, tumor.

[c] Percentage of cells in FNAB/tumor showing 1, 2, 3, 4, or >4 FISH signals, respectively, for the investigated chromosome locus.

of uveal melanoma. The results of our study indicate that the tumor cells obtained by the FNAB are representative of the main tumor, and that the chromosomal aberrations detected by FISH in these FNAB specimens are concordant with the major clonal genetic changes observed by conventional cytogenetics or FISH analysis of the main tumor.

In 249 of 293 hybridizations, we investigated both FNAB and the matched tumor, and statistically, we found a very good agreement between the FISH results of the FNAB and those of the main tumor. Variation between these results was observed for individual probes in 11 hybridizations, a total of 7 cases. However, because more than one probe had been used for the identification of each chromosome, in six of these tumors [EOM-148, EOM-150, EOM-151 (chromosome 8), EOM-157, EOM-171, and EOM-178], the presence of prognostically significant genetic variation could still be established. The results in 2 of 249 hybridizations (0.8%) would have led to the misclassification of the tumor. In EOM-138, we observed only a gain of chromosome region 1p36 in 32% of the main tumor cells and not in the FNAB. Gain of chromosome 1 is a very rare finding in uveal melanoma, and the prognostic relevance of this abnormality is unknown. In EOM-151, only the main tumor showed a subclone with loss of chromosome region 3q24, whereas the centromeric probe for chromosome 3 showed normal copy numbers in both tumor and FNAB samples. In one case, the use of a reference probe or DNA ploidy, *e.g.*, centromeric probes for chromosomes 2, 12, or 13; chromosomes, which are rarely involved in numerical abnormalities in uveal melanoma; or a combined approach of karyotype and FISH analysis could have been helpful to identify the relative loss of chromosomes 1p36 and 3 in a tumor with a hypertriploid karyotype (EOM-125).

The differences between FISH and cytogenetic analysis can be explained by culture artifacts, because the overgrowth of normal cells is not rare when culturing tumor cells. Additionally, FISH has an increased level of resolution for the detection of genetic abnormalities that may not be visible by conventional chromosome studies.

Sisley *et al.* (16) recently demonstrated, using cytogenetic techniques, that all major clonal alterations were detectable in both FNABs and the main tumor. They showed that with short-time cultures of FNABs, conventional cytogenetic analysis was possible in 60% of the cases. The advantage of FISH for identifying genetic variation, as we have demonstrated in the present study, is that it is easier to perform, and the risk of selecting particular cell populations during culture is avoided.

Folberg *et al.* (17) found only a modest correlation ($r = 0.57$) between the SD of the nucleolar area measured in FNABs and that measured in the matched enucleation sample and concluded that the FNABs were unsuitable for determining prognosis using these parameters. However, the differences in sample size between tumor (200 cells/sample) and FNAB (50 cells/sample) in their study could explain their results.

One must keep in mind that these FNABs were performed *ex vivo* under ideal circumstances on relatively large tumors, and we were able to use up to eight probes to compare the FISH results of the FNAB with those of the matched tumor. When these biopsies are taken under less favorable *i.e., in vivo* conditions on tumors planned to be treated with radiation therapy, FISH analysis using probes for chromosome 3 and 8 will be sufficient to predict the outcome of the patient because it is generally accepted that variations of these chromosomes are of high prognostic significance. Furthermore, we[3] and others have shown that these biopsies can also be performed in smaller lesions suspected for uveal melanoma (10), enabling this method to be used in patients preferentially treated with radiotherapy. Our study justifies further studies using *in vivo* biopsies which could enable us to investigate the use of chromosome analysis on FNABs even more accurately.

Until now, no specific regions or genes on chromosomes 3 and 8 involved in the tumorigenesis of uveal melanoma have been identified. However, from our comparative genomic hybridization and spectral karyotyping study, we do have indications that region 8q21.1–21.2/q23–24 and 3q13–21 may be

[3] Unpublished results.

involved (15). Should these regions prove to be the target regions, more specific FISH probes can be designed to improve the predictive outcome of this technique.

Our results demonstrate clearly that FISH analysis of FNAB specimens can be used to examine for the presence of specific chromosomal abnormalities in the tumor cells of uveal melanoma patients. This method is particularly suitable in cases where no tumor tissue has been resected and/or when patients are treated with eye-retaining treatment protocols such as irradiation or radioactive plaque therapy. Detection of these abnormalities provides important additional information for predicting the outcome of these patients and will help to recognize those individuals particularly at risk of developing metastatic disease. The earlier detection of metastases and, if available, the introduction of appropriate adjuvant therapies during primary treatment could contribute to a better survival rate of uveal melanoma patients.

ACKNOWLEDGMENTS

We thank Dr. Caroline Klaver for help with the statistical analysis.

REFERENCES

1. Egan, K. M., Seddon, J. M., Glynn, R. J., Gragoudas, E. S., and Albert, D. M. Epidemiologic aspects of uveal melanoma. Surv. Ophthalmol., *32:* 239–251, 1988.

2. Nathan, F. E., Berd, D., Sato, T., Shield, J. A., Shields, C. L., De Potter, P., and Mastrangelo, M. J. BOLD + interferon in the treatment of metastatic uveal melanoma: first report of active systemic therapy. J. Exp. Clin. Cancer Res., *16:* 201–208, 1997.

3. Woll, E., Bedikian, A., and Legha, S. S. Uveal melanoma: natural history and treatment options for metastatic disease. Melanoma Res., *9:* 575–581, 1999.

4. Mooy, C. M., and De Jong, P. T. Prognostic parameters in uveal melanoma: a review. Surv. Ophthalmol., *41:* 215–228, 1996.

5. Prescher, G., Bornfeld, N., Hirche, H., Horsthemke, B., Jockel, K. H., and Becher, R. Prognostic implications of monosomy 3 in uveal melanoma. Lancet, *347:* 1222–1225, 1996.

6. Sisley, K., Rennie, I. G., Parsons, M. A., Jacques, R., Hammond, D. W., Bell, S. M., Potter, A. M., and Rees, R. C. Abnormalities of chromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis. Genes Chromosomes Cancer, *19:* 22–28, 1997.

7. White, V. A., Chambers, J. D., Courtright, P. D., Chang, W. Y., and Horsman, D. E. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. Cancer (Phila.), *83:* 354–359, 1998.

8. Freire, J. E., De Potter, P., Brady, L. W., and Longton, W. A. Brachytherapy in primary ocular tumors. Semin. Surg. Oncol., *13:* 167–176, 1997.

9. Shields, J. A., Shields, C. L., Ehya, H., Eagle, R. C., Jr., and De Potter, P. Fine-needle aspiration biopsy of suspected intraocular tumors. The 1992 Urwick Lecture. Ophthalmology, *100:* 1677–1684, 1993.

10. Char, D. H., Miller, T. R., and Crawford, J. B. Cytopathologic diagnosis of benign lesions simulating choroidal melanomas. Am. J. Ophthalmol., *112:* 70–75, 1991.

11. Hagemeijer, A., de Klein, A., Wijsman, J., van Meerten, E., de Greef, G. E., and Sacchi, N. Development of an interphase fluorescent in situ hybridization (FISH) test to detect t(8;21) in AML patients. Leukemia (Baltimore), *12:* 96–101, 1998.

12. Luyten, G. P., Naus, N. C., Mooy, C. M., Hagemeijer, A., Kan-Mitchell, J., Van Drunen, E., Vuzevski, V., De Jong, P. T., and Luider, T. M. Establishment and characterization of primary and metastatic uveal melanoma cell lines. Int. J. Cancer, *66:* 380–387, 1996.

13. Hopman, A. H., Ramaekers, F. C., Raap, A. K., Beck, J. L., Devilee, P., van der Ploeg, M., and Vooijs, G. P. *In situ* hybridization as a tool to study numerical chromosome aberrations in solid bladder tumors. Histochemistry, *89:* 307–316, 1988.

14. van Dekken, H., Pizzolo, J. G., Reuter, V. E., and Melamed, M. R. Cytogenetic analysis of human solid tumors by *in situ* hybridization with a set of 12 chromosome-specific DNA probes. Cytogenet. Cell Genet., *54:* 103–107, 1990.

15. Naus, N., van Drunen, E., de Klein, A., Luyten, G. P. M., Paridaens, D. A., Alers, J. C., Ksander, B. R., Beverloo, H. B., and Slater, R. M. Characterization of complex chromosomal abnormalities in uveal melanoma by fluorescent *in situ* hybridization, spectral karyotyping and comparative genomic hybridization. Genes Chromosomes Cancer, *30:* 267–273, 2001.

16. Sisley, K., Nichols, C., Parsons, M. A., Farr, R., Rees, R. C., and Rennie, I. G. Clinical applications of chromosome analysis, from fine needle aspiration biopsies, of posterior uveal melanomas. Eye, *12:* 203–207, 1998.

17. Folberg, R., Augsburger, J. J., Gamel, J. W., Shields, J. A., and Lang, W. R. Fine-needle aspirates of uveal melanomas and prognosis. Am. J. Ophthalmol., *100:* 654–657, 1985.

Clinical Cancer Research

Detection of Genetic Prognostic Markers in Uveal Melanoma Biopsies Using Fluorescence *in Situ* Hybridization

Nicole C. Naus, Anette C. A. Verhoeven, Ellen van Drunen, et al.

Clin Cancer Res 2002;8:534-539.