PRELIMINARY RESULTS OF THE FRO PROJECT: CHROMOSOMAL ABNORMALITIES IN PRIMARY UVEAL MELANOMA.

COOLS D.¹, DEBIEC-RYCHTER M.², PARYS-VAN GINDERDEUREN R.³, HAGEMEIJER-HAUSMAN A.², VAN DEN OORD J.³, SPILLEERS W.¹.

SUMMARY

Uveal melanoma is the most common primary intraocular tumor in adults in the western world. Recent studies indicate that the presence of cytogenetic abnormalities in tumor cells is strongly linked with prognosis. The purpose of this project is the detection of chromosomal abnormalities in a series of frozen tumors (retrospective study) and of fresh tumor tissues (prospective study) and to correlate these results with other prognostic factors and survival.

SAMENVATTING

Uveale melanomen zijn de meest frequent voorkomende tumoren van het oog bij volwassenen. Recent studies hebben aangetoond dat de prognose van deze tumoren sterk gecorrleerd is met de aanwezigheid van cytogenetische afwijkingen. Het doel van deze studie was het opsporen van chromosmale afwijkingen op ingevroren tumorweefsel (retrospectieve studie) en op vers tumormateriaal (prospectieve studie) en deze resultaten te vergelijken met andere prognostische factoren en overleving.

RÉSUMÉ

Chez l’adulte, le mélanome uvéal est la tumeur oculaire la plus fréquente. Des études récentes ont prou-

véd que le pronostic de ces tumeurs est étroitement corrélaté à la présence d’anomalies cytogénétiques. Le but de cette étude était de rechercher des anomalies chromosomales dans des tissus congelés, (étude rétrospective), et dans des tissus frais (étude prospective). Les résultats de cette étude chromosomale seront comparés avec d’autres facteurs pronostiques.

KEY WORDS

Uveal melanoma, chromosomal abnormality, prognosis

MOTS CLÉS

Mélanome uvéal, anomalies chromosomales, pronostic

1 Dienst Oogziekten, St Rafaël UZ Leuven
2 Centrum voor Menselijke Erfelijkheid UZ Leuven
3 Dienst Pathologische Ontleedkunde UZ Leuven

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INTRODUCTION

Uveal melanoma (UM) is the most common form of primary eye cancer in adults in the western world, affecting approximately 6 people per 1 million each year and with a fatal diagnosis in more than 50% of the cases.

At this moment, a number of prognostic factors in uveal melanoma are well established: the histological cell type (4), nucleolar dimension (6), the largest tumor diameter (1), mitotic activity (5), infiltration of the tumor by lymphocytes (10), nuclear proliferating antigen (7), age at presentation (3) and tumor blood vessel morphology (2). All these parameters do participate in the prognosis of the tumor but they cannot yet predict the exact outcome and prevalence of metastatic death. Therefore current research is focusing on mechanisms underlying the metastatic process, including tumor blood vessel morphology, immunology and cytogenetics.

Cytogenetic investigations of UM have revealed that the majority of cases are characterized by recurrent clonal abnormalities involving chromosome 1, 3, 6 and 8 (9) and that the presence of these abnormalities is linked with prognosis (8). Earlier cytogenetic investigations of choroidal and ciliary body melanomas revealed that the majority of cases are characterized by recurrent clonal abnormalities involving chromosomes 1, 3, 6 and 8 (9). More recent studies indicate that the presence of these abnormalities is linked with prognosis. Deviations of chromosome 3 and 8 often occur together, are frequently associated with choroidal tumors and were found to be associated with a poor outcome and thus to be predictors of a poor prognosis.

On the other hand, deviations of chromosome 6 are more frequently associated with choroidal tumors and are predictors for a better outcome (8, 11), even when associated with chromosome 3 and 8 abnormalities.

In our project we are working on cytogenetics and our study consists of two parts: a retrospective study on frozen biopsies and a prospective study on new fresh tissue.

MATERIAL AND METHODS

To perform cytogenetic investigations in our study, we are using 2 different techniques: the Classical Banding technique and the Fluorescence In Situ Hybridization (FISH).

In the Classical Banding technique, a chromosomal chart for karyotyping is performed. With this technique, it is necessary to have sufficient fresh tissue.

The FISH technique gives information on the existence and expression of specific nucleic acids in cells. We make use of probes, cloned and labeled DNA-fragments, which can recognize complemental DNA sequences after hybridization. In this study, 8 different probes were used to demonstrate the presence of chromosomal abnormalities on chromosomes 1, 3, 6 and 8. As compared with the former technique, the FISH technique offers 2 important advantages. It can be applied on fresh or frozen material, because it is not necessary to culture tissue and secondly, a limited amount of material is sufficient for this technique so that Fine Needle Aspiration Biopsy (FNAB) can be used.

Our study consists of 2 parts. Part 1 is a retrospective study of 35 frozen biopsies of uveal melanoma, collected during the period 1989-1995. Frozen tissue is available in the laboratory of histopathology for cytogenetic analysis by FISH technique. These results will be correlated with the clinical outcome and the histopathological findings. The following prognostic parameters will be investigated: the histological cell type, the largest tumor diameter, mitotic activity, age at presentation, tumor blood vessel morphology and scleral invasion. This approach has the advantage of a longer follow-up of the patients.

Up till now, 9 frozen tumors were completely investigated by FISH. (We investigated already 12 frozen biopsies, but in 3 of the cases, the slides contained too few cells for FISH. New slides of these frozen biopsies will be taken in the nearby future.)

Part 2 is a prospective study of new cases during a period of 2 years (1999-2001). We expect to collect about 30 new patients in this period. Large tumors will be studied after enucleation or tumorectomy in 3 different ways.
1) A FNAB of the tumor is taken and a cytogenetic FISH study is performed on fresh cells.
2) A second biopsy is taken and these cells are cultured to perform the classical banding technique with karyotyping as well as the FISH technique on cultured cells. The FISH results of 1) and 2) are then compared to clarify some of the rearrangements and to correctly interpret the FISH data.
3) The bulk of the tumor is then prepared for histopathological investigations.

The cytogenetic results will be correlated with the histopathology of the tumor, with other prognostic factors as well as with follow-up data on 5 years survival.

So far 22 fresh tumors were karyotyped and studied by FISH.

RESULTS AND DISCUSSION

Retrospective study
Up till now 9 cases are completely genetically analysed by FISH. This study showed already that it is well possible to perform this technique on frozen biopsies. Aberrations of chromosome 1 were present in 4 cases, of chromosome 3 in 7 cases, of chromosome 6 were present in 6 cases. In all the cases we found abnormalities of chromosome 8.

The clinical outcome of all the 35 patients has been collected.

Prospective study
Up till now, 22 fresh tumors were karyotyped (classical banding technique) and studied by FISH. Abnormalities of chromosome 1 were present in 5 cases, of chromosome 3 in 11 cases, of chromosome 6 in 7 cases and in 18 cases, aberrations of chromosome 8 were present.

Statistical correlation between the cytogenetic results and the histopathologic and clinical findings will be made in the nearby future, for both the retrospective and prospective part. At this moment the number of cases is to small for statistical analysis.

In this study, we have already proven that cytogenetic analysis by FISH appears to be a valuable tool, applicable to fresh but also to frozen tumors. Multiple recurrent abnormalities were found in all but one case. Correlation of specific abnormalities with survival and late recurrence are under further investigation.

The purpose of this research is the use of the genetic information to determine whether the patient should receive adjuvant chemotherapy and immunotherapy that is under development.

It would be very interesting if the genetic results could predict which patient would benefit from receiving adjuvant therapy.

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Address for correspondence: 
D. Cools 
Dienst Oogziekten 
U.Z. Sint-Rafaël 
Kapucijnenvoer, 33 B-3000 Leuven