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Isonucleolinosis in Cell Cultures of Human Meningiomas

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SUMMARY

In cell cultures of 13 human meningiomas the internal structure of the nucleolus was stained by the toluidine blue-molybdate method and compared with the karyotype of the tumors.

Although some of the meningiomas had lost or gained one or more chromosomes and had undergone structural aberrations, all of them showed isonucleolinosis, which is normally found only in cells with normal karyotype.

It seems possible that the occurrence of iso- or anisonucleolinosis is not a specific sign of euploidy or aneuploidy, but of benignity or malignancy of the examined tissue.

INTRODUCTION

The internal structure of the nucleolus can be demonstrated by the TBM[1] method developed by Love (2) and Love and Walsh (7). With this staining method 2 types of ribonucleoprotein can be distinguished in the nucleolus. The 1 type occurs in the form of roughly spherical structures which correspond to certain nucleolar vacuoles in the living cell (8). These vacuoles were well known to the classical cytologists who termed them "nucleolini" (12). The other type of ribonucleoprotein surrounds the nucleolini and is called "pars amorpha" or "body of the nucleolus" (8).

In a large number of mammalian cell cultures, it was observed that the nucleolini of normal cells were of regular size and shape and distributed uniformly over the whole nulceolus, whereas in neoplastic or transformed cells the nucleolini show great differences in size and distribution (4). For these 2 types of nucleolini, the designation "isonucleolinosis" and "anisonucleolinosis" were offered (3). Meanwhile, this difference in the internal structure of the nucleolus is used in tumor diagnosis (5, 6).

The cytogenetic examination of cell cultures used for the toluidine staining showed that in the case of a normal karyotype isonucleolinosis appeared, whereas in aneuploid cultures anisonucleolinosis was always found. However, a direct correlation between the grade of aneuploidy and anisonucleolinosis could not be established (9).

For a more detailed examination of the connections between the occurrence of anisonucleolinosis and aneuploidy, the human meningioma seems to be very suitable because its chromosomal constitution has been examined in great detail (10, 11, 14-17). It was found that most of the human meningiomas show a loss of Chromosome 22, often combined with the loss of further chromosomes. In some cases, however, a normal or a hyperdiploid karyotype was observed; therefore we had an opportunity to test whether there are differences in the nucleolar structure of meningiomas with normal, hypodiploid, or hyperdiploid karyotype. A previous study indicated that there appears to be a correlation between the loss of acrocentric chromosomes and the number of nucleoli and meningiomas (18).

[1] The abbreviation used is: TBM, toluidine blue-molybdate.

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MATERIALS AND METHODS

Altogether, 13 meningiomas were examined; 4 cultures were grown from fresh biopsies and 9 were from cells that had been stored in liquid nitrogen. Monolayers were cultured on glass coverslips in roller tubes. For toluidine staining, coverslips were removed between the 6th and 14th day of culture, depending on the cell growth. One day before the coverslips were removed, the medium was changed to make sure that anisonucleolinosis did not occur because the cells had entered the stationary phase of culture, as described by Love and Walsh (9). All meningiomas were diagnosed by neuropathologists as nonmalignant. Six were of the endothelial type, 4 were fibromatous, and 3 showed endothelial and fibromatous components. The neurosurgeons reported that none of the meningiomas showed invasive growth.

In addition to TBM staining, chromosome analysis was done on other coverslips of the same cells. For details concerning the culture technique and chromosome preparation, see the papers of Singer and Zang (14), and Zankl and Zang (18). In 9 tumors chromosome fluorescence banding could be obtained by the method of Kim *et al.* (1). The cytogenetic description of chromosomal aberrations followed the Paris nomenclature (1971) as far as banding patterns were concerned. The karyotype of the other tumors is described according to the London Conference (1963) nomenclature.

TBM staining was performed according to the method of Love *et al.* (5). The type of nucleolini was diagnosed by 2 independent investigators after examination of at least 100 cells. The diagnosis of isonucleolinosis was made only if more than 90% of the cells in a tumor showed nucleolini of similar size and even distribution.

RESULTS AND DISCUSSION

The karyotypes of the 13 satisfactorily stained meningiomas are summarized in Table 1. We have purposely included meningiomas with very different karyotypes. Some of them showed a normal chromosome set, others had lost or gained 1 or more chromosomes, and others had undergone structural aberrations. In spite of these chromosomal

Table 1
Data of meningiomas stained with toluidine blue

No. and karyotype	Culture method[a]	Days of culture	Dye concentration (mg/100 ml)
1215 44,XX,−22,−18/45,XX,−22	B	7	20
1409 44,XX,−G,−D	B	7	17
1421 46,XX,+C,−D,Cq+	B	10	15
1423 46,XY,t(4:1)(q2.1;3.2)	B	7	17
1453 44,XX,−G,−E/43,XX,−G,−E,−A	B	5	15
1508 45,XX,−22,19q−/44,XX,−22,−19	B	14	20
1512 52,XO,−22,+5,+7,+9,+11,+15,+17,+19,+20	B	7	20
1530 47,XX,−22,+7,+9	B	8	20
1566 42,XX,−G,−E,−2C/46,XX	B	8	20
1768 43,OY,−22,−14,−16,+mar/ 42,OY,−22,−10,−11,−14,−15,−16,+3mar	A	6	17
1780 46,XX	A	7	17
1781 38,XO,−22,−4,−9,−10,−13,−14,−7,−21,+mar, +p−	A	5	17
1788 46,XX	A	8	17

[a] A, cultured directly from a fresh biopsy; B, deep frozen in liquid nitrogen and cultured after thawing.

abnormalities, all tumors showed isonucleolinosis (Fig. 1 *a* to *d*). This observation stands in contrast to the findings in many other tumors and transformed cell lines, which all showed aneuploidy combined with anisonucleolinosis (Fig. 1 *e*). It was also thought that anisonucleolinosis may be linked to abnormalities of the acrocentric nucleolus-organizing chromosomes. However, in this study several tumors lacked some acrocentric chromosomes and were isonucleolinar. However, none of the tumors lost all the acrocentric chromosomes, so that isonucleolinosis may be dependent on the presence of 1 or more acrocentric chromosomes. It seems likely that the arrangement of the ribonucleoproteins to form iso- or anisonucleolini depends not so much on the chromosome complement, but more on the malignancy or benignity of the tissue examined. Since nearly all malignant tumors show chromosomal aberrations, this may be misinterpreted as a sign of malignancy. Some benign tumors, and especially the meningioma, however, prove this to be wrong, because these tumors show chromosomal aberrations without showing the other criteria of malignancy. Anisonucleolinosis, however, seems to be a specific sign of malignancy and therefore it does not appear in benign tumors. A more indirect correlation between the karyotype and the type of nucleolini is furthermore indicated by the fact that the grade of chromosomal aberration does not influence the proportion of the cells with anisonucleolinosis. For example, the cell line RPMI 2650 which was cultivated from a pleural effusion of a cancer patient (13) showed only a reciprocal translocation between 2 chromosomes, but anisonucleolinosis was very pronounced (3). Cell lines that arose from virus-transformed cells showed a significantly lower rate of anisonucleolinosis than cells from malignant tumors.

The definitive chromosomal aberrations are not mentioned in the review of Love (3), and therefore it is unknown whether transformed cell lines have undergone less severe aberrations of the karyotype than have malignant cells.

It seems necessary to examine other benign tumors with the toluidine blue method to determine whether the results obtained from meningiomas can be generalized for all benign tumors.

REFERENCES

1. Kim, A., Bier, L., Majewski, F., and Pfeiffer, R. A. Fluorochromierung menschlicher Chromosomen mit Atebrin-Essigsaure. Humangenetik, *12:* 257-260, 1971.
2. Love, R. Improved Staining of the Nucleoproteins of the Nucleolus. J. Histochem. Cytochem., *10:* 227, 1962.
3. Love, R. Differences in the Internal Structure of Nucleoli of Diploid and Non-diploid Transformed or Neoplastic Cells *in Vitro*. Exptl. Cell Res., *40:* 188-192, 1965.
4. Love, R. Anisonucleolinosis in Mammalian Cell Cultures. Natl. Cancer Inst. Monograph, *23:* 167-180, 1966.
5. Love, R., Takeda, M., and Soriano, R. Z. Nucleolar Structure in Cancer and Its Diagnostic Value. Ann. Clin. Lab. Sci., *4:* 131-138, 1974.
6. Love, R., Takeda, M., Soriano, R. Z., and McCullough, L. B. The Value of the Internal Structure of the Nucleolus in the Diagnosis of Malignancy. Acta Cytol. *17:* 310-315, 1973.
7. Love, R., and Walsh, R. J. Studies of the Cytochemistry of Nucleoproteins. II. Improved Staining Methods with Toluidine Blue and Ammonium Molybdate. J. Histochem. Cytochem., *11:* 188-196, 1963.
8. Love, R., and Walsh, R. J. The Relation of Nucleolini to Vacuoles in the Living Cell. Exptl. Cell. Res., *53:* 432-446, 1968.
9. Love, R., and Walsh, R. J. Nucleolinar Morphology in Normal Diploid, Neoplastic, and Aneuploid Cells *in Vitro*. Cancer Res., *30:* 990-997, 1970.
10. Mark, J. Chromosomal Patterns in Human Meningiomas. European J. Cancer, *6:* 489-498, 1970.
11. Mark, J., Levan, G., and Mitelman, F. Identification by Fluorescence of the G Chromosome Lost in Human Meningiomas. Hereditas, *71:* 163-168, 1972.
12. Montgomery, T. H. Comparative Cytological Studies with Special Regard to the Morphology of the Nucleolus. J. Morphol. *15:* 265, 1899.
13. Moorhead, P. S. Human Tumor Cell Line with a Quasi-diploid Karyotype (RPMI 2650). Exptl. Cell Res., *30:* 190-196, 1965.
14. Singer, H., and Zang, K. D. Cytologische und cytogenetische Untersuchungen an Hirntumoren. I. Die Chromosomenpathologie des menschlichen Meningeoms. Humangenetik, *9:* 172-184, 1970.
15. Zang, K. D., and Singer, H. Chromosomal Constitution of Meningiomas. Nature, *216:* 84-85, 1967.
16. Zankl, H., Singer, H., and Zang, K. D. Cytological and Cytogenetical Studies on Brain Tumors. II. Hyperdiploidy, a Rare Event in Human Primary Meningiomas. Humangenetik, *11:* 253-257, 1971.
17. Zankl, H., and Zang, K. D. Cytological and Cytogenetical Studies on Brain Tumors. IV. Identification of the Missing G Chromosomes in Human Meningiomas as No. 22 by Fluorescence Technique. Humangenetik, *14:* 167-169, 1972.
18. Zankl, H., and Zang, K. D. The Role of Acrocentric Chromosomes in Nucleolar Organization. I. Correlation between the Loss of Acrocentric Chromosomes and a Decrease in the Number of Nucleoli in Meningioma Cell Cultures. Virchows Arch. Abt. B Zellpathol. *11:* 251-256, 1972.

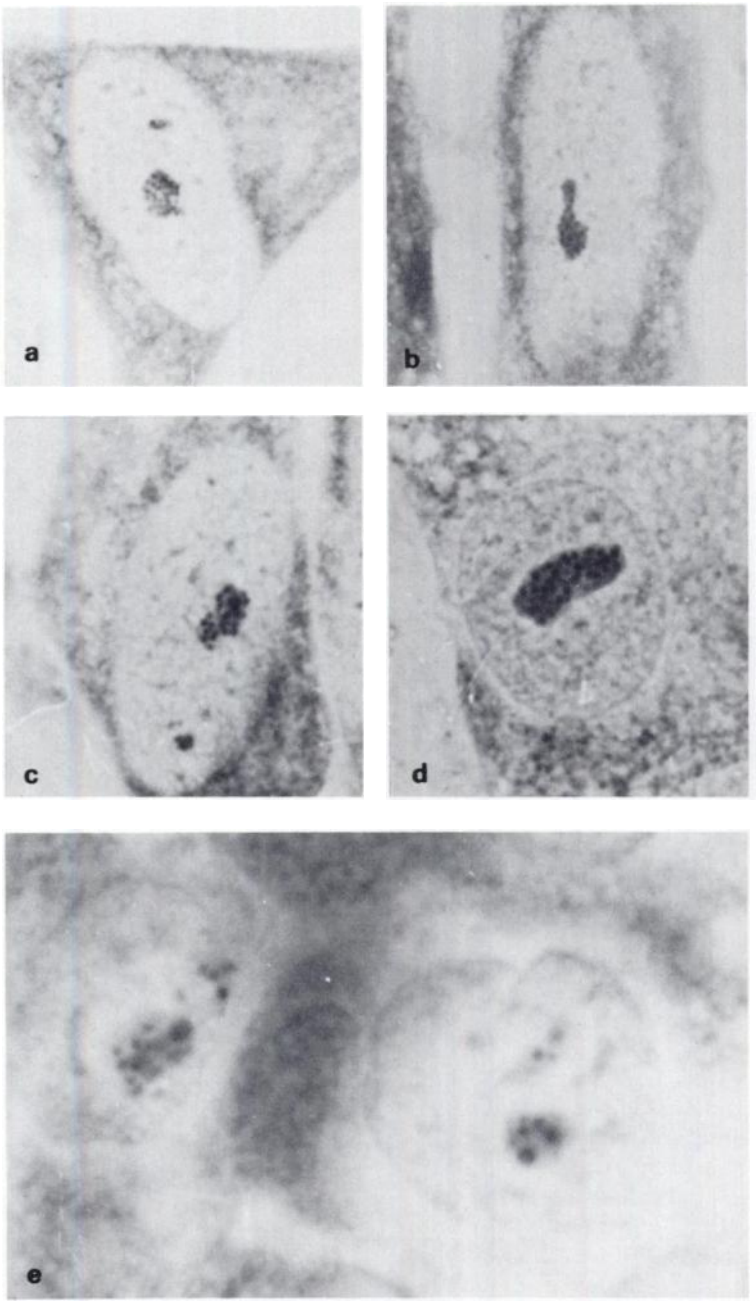

Fig. 1. *A* to *D*, nucleoli of meningioma 1780 (*A*); 1215 (*B*); 1530 (*C*); and 1768 (*D*), all showing isonucleolinosis. *E*, anisonucleolinar nucleoli of carcinoma of the colon *in vitro*.

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