these results are compared with data reflected in the maps of plasmids pAP18-1 and pAP18-ldrd [4] it can be postulated that a change in the incompatibility group Inc FXI of plasmid pAP18-1 to the Inc FVII group in its derepressed mutant is linked with the functioning of different inc genes, located in different regions of the genomes of these plasmids.

LITERATURE CITED

1. A. I. Korotyaev and T. A. Malysheva, Antibiotiki, No. 2, 148 (1988).
2. A. P. Pekhov, Plasmids of Bacteria [in Russian], Moscow (1986).
3. V. P. Shchipkov, N. I. Buyanova, and A. P. Pekhov, Antibiotikii, No. 1, 28 (1986).
4. V. P. Shchipkov, N. I. Buyanova, E. V. Shcherbakova, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 2, 191 (1988).
5. D. L. Daniels, I. R. De Wet, and F. Blattner, J. Virol., **33**, 390 (1980).
6. N. Datta, R-Factors. Drug Resistant Plasmid, ed. by S. Mitsuhashi, Tokyo (1977), pp. 225-272.
7. N. Datta, Plasmids of Medical, Environmental and Commercial Importance, ed. by K. N. Timmis and A. Puhler, Amsterdam (1978), pp. 3-12.
8. T. Maniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning (a laboratory manual), Cold Spring Harbor (1982).
9. R. B. Meagher, R. C. Tait, M. Betlach, and H. B. Boyer, Cell, **10**, 521 (1977).
10. C. Sasakawa, N. Takamatsu, H. Danbara, and M. Yoshikawa, Plasmid, **3**, 116 (1980).

DIAGNOSIS OF ANEUPLOIDY BY IN SITU HYBRIDIZATION: ANALYSIS OF INTERPHASE NUCLEI

S. G. Vorsanova, Yu.B. Yurov, G. V. Deryagin, I. V. Solov'ev, and G. A. Bytenskaya

UDC 616-092:[612.6.05:575.224.23]-076.5

KEY WORDS: interphase nucleus; chromosome number; DNA-probe

Recent advances in genetics have enabled a fundamentally new approach to be made to the diagnosis of hereditary pathology and, in particular, of chromosomal anomalies. This has become possible through improvements in the technology of obtaining recombinant DNA molecules and using them as DNA-probes for the diagnosis of inherited defects. The discovery of chromosomal pathology is based on the use of cloned fragments of the human genome of a special type, namely chromosome-specific DNA probes. With the aid of these probes several distinguishing features of the chromosomal set can be discovered without direct cytogenetic analysis: by DNA hybridization in situ or blot-hybridization [1, 5].

The method of in situ hybridization for detection of chromosomal pathology has an important advantage over other methods of molecular research, in that it does not require a large quantity of biopsy material, isolation of DNA from the patient's cells, the use of the unstable and biologically dangerous isotope of radioactive phosphorus, and the use of the laborious process of blot hybridization. By the use of this method it is possible also to detect and analyze individual chromosomes at virtually every stage of the cell cycle, including in interphase [2, 6], which is particularly important for prenatal diagnosis, when, on the basis of a small volume of material (in native preparations of chorionic cells), it is possible to determine quickly and accurately the sex of the fetus or to discover aneuoploidy.

Moscow Research Institute of Pediatrics and Child Surgery, Ministry of Health of the RSFSR. All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. E. Vel'tishchev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 413-415, October, 1991. Original article submitted September 4, 1990.

0007-4888/91/0010-1480$12.50

TABLE 1. Ratio of Frequencies of Nuclei with Different Numbers of Clusters

Variant of experiment	Karyotype	Number of clusters			
		1	2	3	4
1	46,XX	180(8,6)	1815(87,1)	43(2,1)	46(2,2)
2	46,XX	605(8,4)	6438(89,5)	85(1.2)	64(0.9)
3	46,XX	150(6,2)	2145(88,1)	72(2,9)	67(2,8)
4	46,XX	55(6,6)	725(87,7)	18(2,2)	29(3,5)
5	46,XY	1080(97,6)	18(1,6)	—	—
		8(0,7)*	—	—	—
	46,XY	1130(97,2)	22(1,9)	—	—
		11(0,9)*	—	—	—

Legend. Here and in Table 2, asterisk indicates nuclei with larger diameter and with a single cluster of large diameter; numbers in parentheses are percentages.

TABLE 2. Mean Values of Diameters of Nuclei and Number of Grains of Silver above them, Depending on Number of Clusters (M ± m)

Variant of experiment	Karyotype	Number of clusters			
		1	2	3	4
1	46.XX	8,9±0,3 / 19,8±0,9	10,2±0,4 / 26,5±0,9	13,7±0,5 / 41,0±1,5	16,3±0,6 / 48,7±1,8
2	46.XX	9,6±0,3 / 35,1±1,5	9,6±0,4 / 35,3±1,7	13,3±0,3 / 42,6±2,6	14,6±0,4 / 47,3±2,1
3	46.XX	9,6±0,3 / 21,0±1,0	10,1±0,4 / 25,9±1,1	12,7±0,5 / 55,9±1,7	14,5±0,5 / 65,4±1,8
4	46.XX	9,1±0,3 / 33,9±1,8	10,0±0,4 / 36,0±1,8	13,2±0,3 / 48,6±2,2	16,0±0,4 / 61,6±2,3
5	46.XY	11,7±0,5 / 13,2±0,7	19,3±0,5 / 27,2±1,1	—	—
		19,0±0,7* / 23,6±1,2	—	—	—
6	46.XY	12,0±1,0 / 16,0±0,7	20,4±1,1 / 26,1±0,7	—	—
		19,4±0,5 / 25,6±0,7	—	—	—

Legend. Numerator gives diameter of nuclei (in conventional units); denominator gives number of grains of label.

Research workers frequently are confronted by the problem of possible evaluation of chromosomal anomalies connected with differences in arrangement of the chromosomes (associations, superpositions) in the interphase nucleus. The aim of the present investigation was accordingly to assess the possibility of using in situ hybridization in order to determine the number of X chromosomes in interphase nuclei.

EXPERIMENTAL METHOD

Cultured interphase peripheral blood lymphocytes from patients, sent for psychogenetic diagnosis, and also uncultured cells of chorionic villi, obtained following medical abortions in the first term of pregnancy, were used. The lymphocytes were cultured in Eagle's medium with 20% bovine serum. Chromosomal preparations of the same cultures were obtained by the standard method [3], and cytological native preparations of chorionic villi were obtained by the squash method [2]. Altogether 14,806 cells from four adults and two abortions were analyzed.

For the molecular-cytogenetic investigation we used a cloned probe of pYAM 10-40 α-satellite human DNA, specific for the juxtacentromeric region of the X chromosome [7]. The DNA probes were labeled by the nick-translation method [4]. In situ hybridization was carried out by the method in [4], including denaturation of the preparations in 0.07 N NaOH for 30 sec, hybridization for 18 h, rinsing under standard conditions, and exposure beneath the emulsion for 5 days. The analysis was carried out under an optical microscope with total magnification of 1250 times.

EXPERIMENTAL RESULTS

The ratio of the frequencies of nuclei with different numbers of clusters of granules of radioactive label was determined in all the test samples, and the number of grains of silver in each cluster also was determined in small samples of cells, and the diameter of the nucleus was measured with an ocular micrometer. It was natural to expect that two clusters would be found in the nuclei of cells with the 46.XX karyotype, and that the 46.XY sex constitution would correspond to a single cluster. Analysis showed, however, that besides these numbers, others also were frequently seen (Table 1). It can be tentatively suggested that the doubled number of clusters is the result of polyploidization and is characteristic of tetraploid cells. It can also be postulated that in some cases fusion of the clusters takes place inside the nucleus. Experimentally verifiable consequences stem from these hypotheses.

It is evident that the volume of tetraploid nucleus ought to be twice the volume of the diploid nucleus. Hence it follows that the diameter of the tetraploid nucleus ought to be about 26% greater than the diameter of the diploid nucleus; if, however, we assume a twofold difference not in the volumes, but in the area of the nuclei, the differences in the diameters ought to be about 41%. During fusion of two clusters, the number of granules in the combined cluster thus obtained ought to be about equal to the sum of the granules in the two original clusters, i.e., roughly twice the number of granules in the cluster corresponding to a single chromosome. This latter ratio, however, may be disturbed by the fact that clusters of different sizes have different probabilities of fusion, and the appearance of grains of label takes place with different probabilities depending on how many grains are already present (fusion of grains). Moreover, the probability of fusion of clusters in nuclei of smaller volume is evidently higher than in nuclei of larger volume, and for that reason the mean diameter of nuclei with fused clusters ought to be rather less than the diameter of nuclei without fusion of clusters.

Analysis of the experimental results showed (Table 2) that the values observed are generally in agreement with those predicted by the model, although this agreement is by no means absolute. In fact, the difference in the diameter of the nuclei as a rule was greater than that expected. This can perhaps be explained on the grounds that the difference in the diameters of diploid and tetraploid nuclei by 26% is calculated on the assumption that the nucleus is a sphere; if, however, it is assumed that during preparation of the specimen, the nuclei are flattened to about equal thickness, the difference in the diameters may be expected to be of the order of 41%.

The mean number of grains above the nucleus varies within very wide limits, and sometimes differs quite appreciably from the expected value; here also, however, despite a few exceptions, in general the predicted rule is obeyed, whereas quantitative relations may be disturbed by the qualitative nature of autoradiographic analysis.

Analysis of the distribution of grains of silver among clusters by the chi-square method showed that in all cases clusters with an even number contain about equal numbers of grains – deviations from this rule were not significant in any case. Two hypotheses were tested by analysis of nuclei with three clusters: one – concerning the presence of an equal number of grains in all clusters, the other – the ratio of the number of grains in the clusters (2:1:1) is the result of fusion of two clusters of grains in a tetraploid nucleus. The first hypothesis in all four cases did not agree with the experimental data; meanwhile the observed distributions of the number of grains among clusters are in full agreement with the second hypothesis. It can thus be concluded from the statistical analysis that all consequences of the hypothesis that the cell population includes tetraploid nuclei, and that individual clusters in some cells have fused are in full agreement with the situation observed. Consequently, if this phenomenon is taken into account, the number of X chromosomes in interphase nuclei can be reliably determined by analysis of interphase nuclei. It can be tentatively suggested that with the use of a different molecular probe, similar results can also be obtained by determining the number of other chromosomes in interphase nuclei. Moreover, the statistical analysis showed that in order to determine the number of chromosomes (with particular reference to the X chromosomes) reliably, if mosaicism is disregarded and the probability of an incorrect conclusion does not exceed 0.01, it is sufficient to analyze at least six nuclei.

LITERATURE CITED

1. Yu. E. Vel'tishchev, O. D. Viduta, A. A. Ananenko, et al., Vopr. Okhr. Mat., No. 12, 41 (1987).
2. S. G. Vorsanova, Yu. B. Yurov, I. A. Aleksandrov, et al., Pediatriya, No. 1, 76 (1989).
3. A. F. Zakharov, V. A. Benyush, N. P. Kuleshov, and L. I. Baranovskaya, Human Chromosomes: Atlas [in Russian], Moscow (1982).
4. Yu. B. Yurov, Byull. Éksp. Biol. Med., No. 4, 595 (1984).

5. V. A. McKusick, Human Gene Mapping, No. 10, New Haven (1989).
6. S. G. Vorsanova, Yu. B. Yurov, I. A. Aleksandrov (I. A. Alexandrov), et al., Hum. Genet., **72**, 185 (1986).
7. Yu. B. Yurov, A. G. Yakovlev, I. A. Aleksandrov (I. A. Alexandrov), et al., Cytogenet. Cell Genet., **51**, 1114 (1989).

USE OF MONOCLONAL ANTIBODIES AGAINST MYOSIN LIGHT CHAINS 1 TO DETECT THE CORRESPONDING ANTIGEN IN BLOOD OF PATIENTS WITH MYOCARDIAL INFARCTION

E. V. Pulyaeva, A. L. Konorova, L. I. Kovalev, A. Yu. Volgin, S. S. Shishkin, and L.A. Pevnitskii

UDC 616.127-005.8-07: 616.153.96-097-078.33

KEY WORDS: myosin light chain 1; monoclonal antibodies; polyclonal antibodies; myocardial infarction

The human myocardium, an example of highly differentiated tissue, is characterized by a very specific set of contractile proteins. It has been shown, in particular, that expression of a special active gene takes place in heart muscle, and this is accompanied by the corresponding synthesis of a tissue-specific form of actin [7]. By contrast with this, the tissue-specific form of α-tropomyosin is formed, not through expression of a special gene, but by means of alternative splicing of pre-mRNA of the gene which determines synthesis of the α-tropomyosins of skeletal and smooth muscles [6]. Myosin light chains 1 (MLC1) also are tissue-specific, and even the existence of different isoforms of MLC1 has been demonstrated in the myocardium of the ventricles and atria [3].

Previously the writers described obtaining a hybridoma clone, producing antibodies which "recognize" ventricular MLC1 of human heart muscle, but do not react with myosin light chains from other tissues [2]. Degenerative changes in the myocardium (especially infarction) may be accompanied by the appearance, not only of cytosolic enzymes of heart muscle, but also of certain contractile proteins, in the blood plasma [5].

In this investigation an attempt was made to use monoclonal antibodies obtained previously to look for the corresponding antigen in the blood plasma of patients with myocardial infarction, for it might become a highly specific marker of myocardial dystrophy.

EXPERIMENTAL METHOD

The test object consisted of samples of venous blood from four patients with myocardial infarction, obtained in the active phase of the disease. The diagnosis in all cases was based on EEG data and determination of transaminase activity and other biochemical tests. Blood samples from four healthy individuals served as the control. The cells were separated from the plasma by centrifugation. Preparations of plasma were used for enzyme immunoassay (EIA). Monoclonal antibodies produced by clone MLC-1c were obtained as described previously [2]. Polyclonal antibodies against MLC1 of the human ventricular myocardium were prepared from serum of rabbits immunized with the corresponding antigen by the method in [4]. The content and specificity of polyclonal antibodies were verified by carrying out immunoblotting of the zone of myosin light chains by two-dimensional electrophoresis of extracts of human heart muscle [2]. The antibody titer was determined in

Research Institute of Human Genetics, All-Union Medical Genetics Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 416-417, October, 1991. Original article submitted February 15, 1991.

