CHROMSOME IMBALANCE AND PROLIFERATIVE POTENTIAL OF CELLS *in vitro*

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UDC 618.33-055.5.7-07:616-018.1

KEY WORDS: chromosome imbalance; human fibroblasts; cloning effectiveness.

The effect of chromosomal imbalance on cell replication *in vivo* is not yet understood. It has been suggested that phenotypic features connected with anomalies of this kind (monosomy, trisomy) may be due to disturbance of the ability of cells to multiply [10]. It has been shown that in some anomalies the parameters of the cell cycle are altered [9]. However, the results of investigations on cells in culture are contradictory. Some workers have observed reduced ability of cells to multiply [4, 11], whereas others deny any connection between chromosomal pathology and proliferative characteristics of cells [6, 8]. The contradictoriness of these data may be explained on the grounds that precise parameters of cell proliferation have been used in only a few investigatons. In others, the main task of which was to identify the karyotype in spontaneous abortions, cell proliferation parameters were rather subjective: for example, counting the number of days of culture needed to obtain a sufficient number of cells for cytogenetic analysis [6]. The nonobjectivity of evaluation of cell proliferation may lead to contradictions.

The percentage of cells capable of forming colonies consisting of 16 cells or more may be a sufficiently reliable parameter of the proliferative potential of a strain. Highly significant correlation has been demonstrated between this value and the doubling potential of the cells — the "lifetime of the strain" [13]. The aim of the present investigation was accordingly to study the effect of chromosomal imbalance on the proliferative potential of the cells.

EXPERIMENTAL METHOD

Experiments were carried out on 43 strains of human skin fibroblasts: 16 strains were obtained from medical abortions at 8-12 weeks, 15 strains from healthy donors aged 25-35 years (these strains possessed a diploid set of chromosomes), seven strains were obtained from spontaneous abortions at the 6th-12th week of pregnancy (trisomy for chromosomes 7, 9, and 14, monosomy for chromosome 21, triploid set of chromosomes), and five strains were obtained from patients with Down's syndrome, Turner's syndrome, and poly-X syndrome.

The cells were cultured in a mixture of: Eagle's medium 85%, bovine serum 10%, human umbilical serum 5%. The ratio of these components for cell cloning was somewhat different: 80, 10, and 10% respectively. The conditions for trypsinization of the cells and conduct of cloning efficiency experiments (percentage of cells capable of forming colonies consisting of 16 cells of more) were described previously [3]. Since one of the main factors influencing cell proliferation is the serum component of the nutrient medium, batches of bovine serum and human cord serum selected beforehand were frozen (—20°C) and thawed immediately before the experiments. Sera were selected on the basis of their high ability to maintain clone formation of the cells. All experiments were carried out on the same batches of medium and serum. Each value of cloning efficiency was the mean of 15-30 measurements. Statistical analysis of the results by the HP-9815A computer (USA) showed that the distribution of individual values of this parameter obeys the law of a normal distribution. The error for each mean value of cloning efficiency did not exceed ±3% (level of significance $P < 0.05$). All strains were studied in 5-10 culture passages.

EXPERIMENTAL RESULTS

Data on cloning efficiency are given in Fig. 1. Cloning efficiency of strains obtained from medical abortions lay within the interval 38-82%. The proliferative potential of cells

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 2, pp. 191-193, February, 1985. Original article submitted February 22, 1984.

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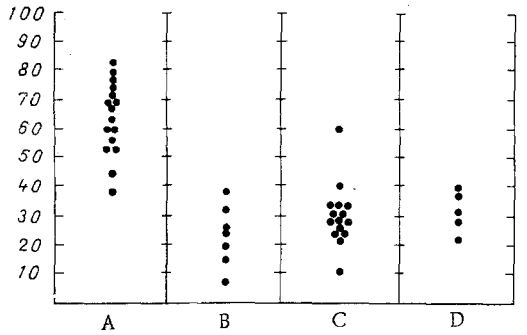

Fig. 1. Proliferative potential of cells from normal subjects and in hereditary pathology. A) Embryonic diploid strains, B) embryonic aneuploid, C) postnatal diploid, D) postnatal aneuploid strains. Vertical axis — cloning efficiency of strains (in %).

obtained from spontaneous abortions with chromosomal imbalance was sharply depressed compared with that of cells from medical abortions. Significant differences ($P < 0.01$) in cloning efficiency were found (5, 20, and 31%) between these strains obtained from different spontaneous abortions, but with the same karyotype (47XY + 7).

The cloning efficiency of postnatal strains obtained from healthy donors was between 12 and 60%. Of 15 strains, two had cloning efficiency over or equal to 40%, one strain had very low efficiency (12%), and the remaining 12 strains showed closely similar values (20-35%). Chromosomal imbalance in postnatal cells did not lead to a decrease in proliferative potential compared with the control.

In all cases cloning efficiency was reproduced closely from one experiment to another and was a characteristic of each individual strain.

Phenotypic manifestations of chromosomal anomalies are known to be highly variable. For instance, trisomy 13 or 18 may lead to termination of development of the embryo [2, 4], to the birth of a child with a series of developmental defects, described as Patau's and Edwards' syndromes respectively, or they may have minimal phenotypic manifestations [7]. The phenotypic effect of chromosomal imbalance evidently depends on the genotypic background and on external environmental factors. However, this statement, while undoubtedly true in its essentials, is too general in character. In man, the investigator virtually cannot estimate the "genotypic background" and take into account external environmental factors modifying the effect of chromosomal imbalance. A solution to this problem may be facilitated, in our opinion, by identification of individual variability of the most important morphogenetic processes under genetic control. One such process in our investigations is cell proliferation, assessed as cloning efficiency. The high reproducibility of this parameter for individual strains is evidence of the important contribution of the genetic component, and the quite considerable differences between this parameter for different strains are evidence of its individual variation in the population. It may accordingly be postulated that the severity of the phenotypic manifestations of chromosomal imbalance may be largely connected with the genetically determined proliferative potential of the cells of the given individual. If cells with a low value of the proliferative potential also have a chromosomal anomaly, this interaction most likely will lead to the severest consequences of morphogenesis, manifested as termination of development in the early stages, i.e., to spontaneous abortion. The high proliferative potential, genetically determined initially, creating a "higher buffer capacity" of this process in relation to proliferative ability, will facilitate the "smoothing" of the phenotypic manifestations of chromosomal imbalance, leading to survival of an individual with a disturbance of this chromosomes. A situation like this is characteristic of lethal mutations in general. Hadorn [5], for instance, pointed out that certain genes and combinations of genes, acting as embryolethal under ordinary conditions, do not have that effect under other conditions. Individuals with a lethal genotype, who avoided death in the early embryonic period for one reason or another, he calls "escapers." However, the presence of more or less severe developmental defects, frequently producing definite syndromes, are characteristic of these "escapers." In the light of these data it can be postulated that chromosomal imbalance, acting against a background of low proliferative cell potential, will be manifested as embryolethal, whereas embryos with genetically determined high proliferative

potential will "escape" death in the embryonic and fetal period, but will have congenital development defects.

Our data show that cells with chromosomal anomalities obtained from patients have values of proliferative potential indistinguishable from normal, whereas embryonic cells with chromosomal imbalance will have a reduced proliferative potential. This is understandable if individuals born with a chromosomal anomaly are considered to be "escapers." In the light of our observations the contradictory nature of data in the literature on proliferation of cells with chromosomal anomalies also becomes understandable. The chromosomal imbalance itself, if it affects proliferation, does so only negligibly; however, its effect may be superposed on variation of this parameter existing in the population, leading to a shift of the distribution toward lower values. This action of trisomy of chromosome 21 on metric parameters was demonstrated by Shaprio [12], who formulated the concept of "branched disturbance of homeostasis."

Proliferative cell potential, of course, is not the only genetically controlled morphogenetic parameter modifying the phenotypic effect of chromosomal imbalance. It is highly that variation in other processes playing the principal role in morphogenesis leaves a definite imprint on the manifestation of chromosomal anomalies. It is important to note that variability of the "buffer capacity" of many key processes of development and, consequently, of their resistance to the damaging action not only of chromosomal anomalies, but also of mutant genes and external environmental factors, exists in the population. It should be noted that this interpretation of our experimental data is in full agreement with the hypothesis of conditonal tropism, elaborated by Davidenko [1] in the 1930s in relation to hereditary diseases of the nervous system.

According to this concept, polymorphism of clinical manifestations of many hereditary diseases of the nervous system is connected with interaction between many genes, creating the variation of features connected with the nervous system that exists in the population, and a "principal" gene, leading to the onset of a definite disease.

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