

Preparation and application of cells used in the external quality assessment of karyotype analysis.

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Abstract

Objective: To study the preparation and application of cells used in the external quality assessment of chromosome karyotyping and use the cells in the external quality assessment.

Methods: After diagnosis of cytogenetics or molecular genetics, and the delivery of diagnostic reports, residual living cells adhered to the culture flask with uncommon and complicated chromosomal abnormalities which were likely to be misdiagnosed were collected. Cells were collected after multiple cell passaging or superfluous cells were collected as the procedure for passaging cells was ongoing. Cells with chromosomal abnormalities of the same kind or the same case which were collected at different time were preserved together after the preparation of cell suspension and fixation of cells. Then cells were mixed together in different proportions and species according to the requirements of the external quality assessment, obtaining different ratios of mixed cell suspensions of cells with abnormal chromosomes and chimeric karyotypes. The cell suspensions were stored for the external quality assessment of chromosome karyotyping.

Results: Cell suspension which contained chimera with 4 types of abnormal chromosome karyotypes was prepared during the trial-produce. The karyotypes contained in the chimera were 46, xy, t (1;20) (q32;p21), 46, xx, t (13;14) (q10;q10), 45, xx, t (13;22) (q10;q10), and 47, xx, +mar. The cell suspension can be delivered to the laboratories and used in the external quality assessment.

Conclusion: The preparation of cells with abnormal chromosomes and chimeric karyotypes and their application in the external quality assessment of chromosome karyotyping make it possible to evaluate chromosomes prepared by laboratories, and to assess the accuracy of diagnosis of chromosome structures and abnormal chromosomes as well as the ratio of the chimeras by laboratories at the same time. It is a feasible method of the external quality assessment.

Keywords: External quality assessment, Quality control agent, Chromosome karyotype analysis.

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Introduction

With the increasing emphasis on reproductive health and population quality in China, in recent years, prenatal cytogenetic diagnosis has been widely used in the diagnosis of chromosomal disorders in prenatal fetus and an embryo prior to the implantation. In addition, prenatal cytogenetic diagnosis serves as an important indicator for the clinical diagnosis and studies on the pathogenesis of diseases, such as abnormal pregnancy and infertility. For laboratory diagnostic projects, there should be standardized laboratory quality control methods, and laboratories should be involved in the external quality assessment organized by the health authorities, which has become a consensus and government behavior [1-3]. Although there are a lot of articles [4-6] which have reported

methods used for the internal quality control and external quality assessment of chromosome karyotyping, operating conditions of the preparation, delivery, chromosome preparation and practical application of the quality control agent have yet to be further optimized. In this paper, the preparation and application of cells used in the external quality assessment of chromosome karyotyping were studied. We believed that the cell with complicated chromosomal abnormalities and chimeric karyotypes is an ideal material in the external quality assessment, and that their application is a simple and convenient method, which is synchronous with chromosome preparation and karyotype analysis.

Materials and Methods

Primary cells with chromosomal abnormalities

Primary cells were derived from residual primary cells or living subculture cells adhered to the culture flask with uncommon and complicated chromosomal abnormalities which were likely to be misdiagnosed, after the chromosome preparation, and diagnosis of karyotype analysis.

Reagents

Reagents used in the conventional cell culture and chromosome preparation included RPMI-1640 medium, 25% fetal bovine serum, 0.25% trypsin, 20 µg/ml colchicine, 0.075 M KCl hypoosmotic, fixation solution (mixture of methanol and glacial acetic acid, 3: 1), and Giemsa stain.

Passaging and clonal expansion of cells

According to the conventional cell passaging method [7], primary cells or living subculture cells adhered to the culture flask were selected. After each 3 to 7 days of culturing, the previous medium was replaced with fresh culture medium after the cells reached about 85% confluency. Trypsin (0.25%) was added to digest adherent cells and cells were then washed with sterile saline. Appropriate amount of medium was added to suspend cells. Then cells were transferred into a relatively large culture flask to conducted cell passaging. The above procedures were repeated for several times until 50 passages were finished. Cells were expanded, which were used in the cell harvesting and chromosome preparation. Or cell subcultures were harvested in the process of passaging.

Preparation of cell suspension

Cells were harvested by conventional method [7], and treated with colchicine, followed by trypsin digestion, hypotonic treatment, and fixation by the mixture of methanol and glacial acetic acid. Cell suspension (10⁵/ml) was obtained, then stored at -20°C.

Preparation of chimeric cells for quality control

The above cell suspension was taken. According to the requirement of the external quality assessment, cell suspension of cells from 1 case was mixed with other cell suspension from 1 to 50 cases with the volume ratio of 1: 1-20 or according to the proportion of cell numbers. The mixed cell suspension contained cells with abnormal chromosomes with different types and proportions, while these cells were considered as quality control cells of the same case of illness.

The establishment of quality control cell bank

The fixed solution (methanol: glacial acetic acid=3: 1) with the concentration of 105/ml was used as the medium, and was loaded into tubes (1 ml/tube). The fixed solution was added to the mouth of the tube, followed by sealing and numbering. The tubes were preserved in a device at -20°C. Meanwhile, the

description of standard karyotypes and other information were imported into the computer management system.

Methods of application

The numbers of quality control cells were extracted from the computer, and then the corresponding quality control cells were found out from the quality control cell bank, and delivered to laboratories involved, according to the literature [8-10]. Each laboratory routinely performed the preparation of chromosome and karyotype analysis, and reported results. Then the misdiagnosis of the external quality assessment was discussed, and the causes were analyzed in order to increase the experience of technical staff and improve the quality of diagnosis.

Results

A cell suspension which contained chimera with 4 types of abnormal chromosome karyotypes was prepared during the trial-produce as shown in Table 1. The karyotypes contained in the chimera were 46, xy, t (1;20) (q32;p21); 46, xx ,t (13;14) (q10;q10); 45, xx, t (13;22) (q10;q10) and 47, xx, +mar. The preparation was successfully and the cell suspension can be delivered to the laboratories involved in the external quality assessment. Under the premise of synchronization with the actual operation of chromosome preparation and Karyotype analysis, the cell suspension can be used to evaluate chromosomes prepared by laboratories, and assess the accuracy of diagnosis by laboratories of chromosome structures and abnormal chromosomes as well as the ratio of the chimeras.

Table 1. Karyotypes contained in the chimera.

Karyotypes
46, xy, t (1;20) (q32;p21)
46, xx ,t (13;14) (q10;q10)
45, xx, t (13;22) (q10;q10)
47, xx, +mar

Discussion

Quality control of experimental diagnostic projects has attracted increasing attention, and has become a government action. A consensus that the quality of experimental diagnostic projects should be controlled has been reached [1-3]. Recently, there have been a lot of reports and literature on the quality control of imaging, cytogenetics, molecular genetic prenatal diagnosis and maternal serum of prenatal screening [11-13]. Bastien et al. [14] performed a 3-year external quality assessment of molecular diagnosis of Toxoplasma in amniotic fluid made by a number of laboratories. Tosto et al. [15] carried out a five years' investigation of genotyping, reported results and test methods of Thalassemia and proposed implementation plan of local external quality assessment. Ramsden et al. [6] reported a 3-year experience of external quality assessment of rapid prenatal detection of numerical

abnormalities of chromosomes using molecular genetic techniques. A study [5] reported the preparation of immortal cell line with abnormal chromosome karyotypes and its application in external quality assessment for chromosome karyotype analysis. This reported method involves the whole process of quality control of cell culture, chromosome preparation, reagents and the interpretation of images. However, further studies are needed on the optimum conditions of preparation of quality control cell lines, delivery of cell lines and chromosome preparation. Following the literature [8-9] which proposed implementation methods of images of complicated abnormal chromosome karyotype in the external quality assessment, several studies in China [10] also proposed the methods of establishment of abnormal chromosome karyotype image library and implementation of chromosome karyotype analysis. These methods have advantages of easy access of images of abnormal chromosomes, costs cost approaching to zero, and easy operation of external quality assessment. However, these methods don't involve the quality control of cell culture, chromosome preparation, and relevant reagents. In addition, the process of image analysis of abnormal chromosomes in the external quality control is not completely synchronized with the actual operation, and doesn't involve the diagnosis of common chimeras.

In order to solve the above problems, we studied the preparation of cells with chimeric karyotypes and its application in the external quality assessment of chromosome karyotyping. Primary cells were derived from residual primary cells or living subculture cells adhered to the culture flask with identified uncommon and complicated chromosomal abnormalities which were likely to be misdiagnosed. According to the literature [7], the cells were expanded repeatedly to increase the number of cells. Generally, diploid cells cultured in vitro can be passaged to 50 or more cell subculture. Then based on the requirement of the external quality assessment, cell suspension of cells from 1 case of illness was mixed with other cell suspension of cells from 1 to 50 cases of illness in the volume ratio of 1:1-20 or in the proportion of cell numbers. The mixed cell suspension contained cells with abnormal chromosomes with different types and proportions. After repeated passaging, cells were collected, and cells with chromosomal abnormalities from different cases were mixed. The number of cells were not only sufficient in quantity, but also the primary cells which contained one type of chromosomal abnormality were changed into quality control cells which contained a variety of misdiagnosed abnormal karyotypes of chromosomal chimeras mixed in different proportions. Thus, on the basis of being prone to misdiagnosis, the difficulty and complexity of identification and diagnosis of chromosomal chimeras were added.

Each of chimeric cells for the external quality assessment prepared in this study contained 4 abnormal karyotypes, 46, xy, t (1; 20) (q32; p21), 46, xx, t (13; 14) (q10; q10), 45, xx, t (13; 22) (q10; q10), and 47, xx, +mar. It is derived from theoretical calculation that the proportion of each type of chimeras was 25% if the equal volumes of 4 types of cell suspensions

containing cells with abnormal chromosomes were mixed. However, in fact, due to in the presence of various factors in the chromosome preparation, the proportions would change. The average of proportions in laboratories participating in the same external quality assessment could be taken as the "target value", which was considered as the exact proportion. Or the selected experienced professionals in the reference laboratories could determine the "target value", that is the proportion of each type of abnormal karyotypes. In the process of external quality assessment, quality control cells were sent to laboratories or specialists in accordance with the literature [8-10]. After the diagnosis of chromosomal abnormalities was completed independently on time and in accordance with the regulations, diagnosis results were fed back. Then according to four-point scale scoring method in the literature [5], the misdiagnosis and missed diagnosis reported by laboratories involved were evaluated. The causes were analyzed, regular discussion was hold, and the existing problems were corrected in order to increase the experience of technical staff and improve the quality of diagnosis.

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