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Potential and Perspectives of a-CGH Method in Clinical Practice

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ABSTRACT

The identification of chromosomal abnormalities is an important component of clinical genetics. Currently, the methods of classical cytogenetics cannot meet the increasing demands of clinical medicine. The development of fluorescence *in situ* hybridization techniques (FISH, CGH) has made possible the identification of submicroscopic chromosome aberrations. Progress of the technology has led to the emergence of new high-tech method - comparative genomic hybridization on microarrays (array-CGH, a-CGH). This method is used in many areas of clinical genetics, reproductive medicine and oncology. Besides practical applications, a-CGH is used in fundamental medicine, particularly to study the causes of congenital malformations and undifferentiated intellectual disability, as well as fundamental oncology.

Keywords: a-CGH, cytogenetic diagnostics, microarrays.

Submicroscopic chromosomal deletions and duplications (less to 10 Mb) constitute up to 15% of all mutations underlying human hereditary diseases [6]. Comparative genomic hybridization on microarrays is the most appropriate method to detect such abnormalities. Resolution of modern microarrays reaches several tens of kb, while up to 1 million unique genomic loci is scanned. The main advantage of the method is extremely high resolution, allows for the identification submicroscopic chromosomal aberrations as well as copy number variations of large blocks of DNA repeats (Copy Number Variation, CNV). CNV accounts for roughly 12% of human genomic DNA. Copy number variation can be inherited from parents or arise *de novo* in any part of the genome, its size can be relatively small – a few hundred thousand base pairs. More than 41% CNV overlap with known genes, indicating their possible role in the regulation of the expression through the effect of gene dose or position [1]. In the future, the analysis of the genes on the chromosome regions affected by submicroscopic chromosomal abnormalities or CNV will help to identify genes with imbalance leading to the development of pathology. Noticeable shift of focus in cytogenetic studies from chromosome to gene, as well as the expected introduction of high-throughput genome sequencing allows the appearance of a new direction in biology and medicine – cytogenomics [2].

Array CGH method is based on the principles of the conventional comparative genomic hybridization (c-CGH). Differentially labeled control DNA (usually labeled with a red fluorochrome) and target DNA (labeled with green fluorochrome) hybridize together on small fragments of human DNA deposited in a specific order to the microchip. Differences between gain and loss or a balanced status are based on the ratio of green to red fluorescence for each DNA fragment. Further, by specific processing programs all DNA segments are positioned on a specific chromosomal region, wherein the hybridization profile generated reflecting the amount of DNA material in each region of the genome.

Microarrays differ in their resolution. Microarrays that use large segments of DNA (100-200 kb) built-in artificial bacterial chromosome (BAC - bacterial artificial chromosome) are applied for low resolution a-CGH. In average, they have a resolution of 1 Mb (i.e. register gain or loss of genetic material quantity of 1 Mb, which roughly corresponds to 1/10 of the chromosomal band). High-resolution microarrays have a resolution of 50-100 kb. They are consisted of the oligonucleotide sequences of about 60 nucleotides in length, relatively homogenously covering the whole genome. For example, available commercial microarrays provide an average coverage of the genome with a resolution up to 0.02 Mb that is 1000 times more informative than karyotyping [3].

Array CGH method allows to identify all unbalanced chromosomal rearrangements, including aneuploidy, unbalanced translocations and microstructural abnormalities in a single analysis. Disadvantages of the method include the inability to detect balanced structural abnormalities (reciprocal translocations, inversions and Robertsonian translocations) and polyploidy [8].

Reproductive medicine is a key area where CGH microarray technology is demanded. Usage of assisted reproductive technology (ART) is accompanied by a high frequency of chromosomal abnormalities in embryo cells. After the application of ART, the frequency of abnormal embryos with chromosomal abnormality in women younger than 35 years is 60%, and for women over 41 – 80% [9]. Preimplantation genetic diagnosis (PGD) can detect abnormal embryos, greatly increasing the chance of successful implantation of the blastocyst and successful delivery. Currently, for purposes of PGD FISH and a-CGH are used. FISH commonly allows identification of aneuploidy for five chromosomes (13, 18, 21, X and Y) only with clinical significance at once, whereas the use of a-CGH allows genome-wide assessment of aneuploidy and unbalanced structural chromosome aberrations.

Listed advantages of microarrays in comparison with FISH remain in prenatal diagnosis. Array CGH in prenatal diagnosis of chromosomal abnormalities is used primarily on the testimony, for example, if the parents are carriers of balanced translocation. In addition, a-CGH is used to identify the causes of spontaneous abortion. So, we investigated 13 miscarriages with normal karyotype determined by conventional cytogenetics. From 3 to 20 CNV were detected in each case. In 4 embryos only benign variants were observed, while 9 abortions had potentially pathogenic CNV.

Microarray technology is most prevalent in areas of clinical genetics such as search for genetic causes of intellectual disability and congenital malformations [4]. This trend is due to the fact that many undifferentiated forms of these pathologies result from various CNV and submicroscopic chromosomal rearrangements. CNV underlie the 14-18% of undifferentiated intellectual disability [5]. Currently, 211 microdeletion and 79 microduplication syndromes are described that cover 267 genomic loci [7]. Research in this field was also conducted by our team. Samples from 79 children with intellectual disability and congenital malformations were analyzed using Agilent 44 K and 60 K arrays. Array CGH analysis did not identify any unbalanced chromosomal aberrations in 35 of the patients (44%). Copy number variations that were observed in the remaining 44 patients were first classified using the Database of Genomic Variants. Twenty-two children carried only benign CNV. A total of 26 pathogenic or likely pathogenic CNV were detected in the other 22 affected children (28%) [4].

Microarray technologies are an integral part of modern cytogenetics. Demand for this method is due to the possibility of simultaneous analysis of the whole genome with an extremely high resolution. Given the significant advances in this area in the last decade, as well as due to ever-lower cost, a-CGH technology in the near future can be widely used in medical practice. Effective implementation of this technology into clinical practice requires not only technical equipment and qualified personnel, but also the maximum awareness of clinicians.

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