Single Fetal Cells for Non-Invasive Prenatal Genetic Diagnosis: Old Myths New Prospective

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INTRODUCTION

Since 1980, prenatal ultrasound and maternal serum markers have been used as non-invasive first and second trimester screening tests for common birth defects, including neural tube defect and Down syndrome. The sensitivity is around 81% to 95% with a false positive rate of around 5% [1,2]. Results often require follow-up invasive confirmatory tests on chorionic villous sampling (CVS) or amniocentesis (AF). Fetal DNA is present in maternal circulation during pregnancy [3]. These fetal DNA can be used for prenatal genetic diagnosis [4]. In 1997 and the following years, Lo and co-workers successfully developed techniques in fetal DNA amplification from maternal peripheral blood, thus implementing new hallmark in non-invasive prenatal diagnosis (NIPD). Advantages of fetal DNA for prenatal genetic diagnosis include non-invasive, fast and reliable. In present market, there are at least four different companies offering NIPD with different approaches, including the MaterniT21™ PLUS from Sequenom, VeriFID™ from Verinata, Harmony™ from Ariosa and Panorama™ from Natera [5]. These tests are originally designed for detection of Trisomy 21 but, in theory, can also detect other aneuploidies including, Trisomy 18, Trisomy 13, and sex chromosome aneuploidies, the common chromosomal abnormalities in live births. The sensitivity ranges from 98.6% to 100% with specificity from 99.8% to 100%. The false positive rate is between 0% and 0.2% subjected to further large-scale verification. However, fetal DNA has its limitations. First, maternal DNA is extracted together with fetal DNA. High maternal DNA background not only restricts the direct fetal genetic test, but also results in higher false positive rate than conventional methods [6]. Second, fetal DNA in maternal circulation is fragmented. Incomplete genetic information limits the NIPD to only small categories of common genetic diseases, such as sex determination, Rh blood group neonatal hemolytic diseases, some single gene disorders and major chromosomal aneuploidies [7,8].

FETAL CELLS FOR NIPD

Fetal cells can be isolated from maternal peripheral blood circulation [9,10]. They include fetal multinucleated white cells, mononucleated white cells, lymphocytic blood cells, red blood cells, nucleated red blood cells and even fetal stem cells. Use of fetal cells facilitates a direct analysis of true fetal genetic information. Although fetal cell populations are very rare, less than 0.1% of total cells in maternal circulation, isolation of fetal cells has invaluable potentials for NIPD as next gold standard (Table 1). If fetal cells can be isolated successfully, the cells are

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purely fetal origin which enhances the accuracy of NIPD without false positive detection. In addition, isolated fetal cells carry non-fragmented whole fetal genome which allows any genetic tests and even functional assays. Recent new technology has emerged to isolate specific cells from peripheral blood such as free circulating cancer cells [11]. The cells possess unique cell size and specific markers which enable them to be tagged with specific antibodies conjugated with either magnetic beads or fluorescent dyes then sorted by various sorting technologies [5]. Several cell isolation and sorting products are available in the market, e.g. magnetic-based Magnetic-Activated Cell Sorting (MACS) technology from many companies, microfluidics-based C1™ Single-Cell Auto Prep System from Fluidigm, DEPArray System from Silicon Biosystems, and optic-based Fluorescence-activated cell sorting (FACS) technology from various companies. The isolation efficiency can be achieved 1 in 100,000 and up to 1,000,000 cells [5]. These new technology and equipments can be applied to fetal cell isolation for NIPD [12].

OPPORTUNITY AND CHALLENGES

With advances in techniques for NIPD, traditional methods are challenged with the developed maternal fetal DNA analysis.

Figure 1 Scheme for isolating fetal cells in maternal blood by cell sorting technologies and subsequent tests for non-invasive prenatal genetic diagnosis.
It was hoped that such techniques may ultimately replace conventional invasive techniques for the benefits of pregnant mothers. Some of these methods can have very high specificity and sensitivity and even very low to zero percent false positive rates, however, these methods do not directly test the fetal cells nor study all the genetic makeup of the fetal tissues, some important genetic diseases or dysfunctional genes may not be revealed readily. Direct NIPD of fetal cells should be better than fetal DNA fragments. New platforms for the isolation and analysis of fetal cells down to a single cell level are available now. Besides, complete and extensive studies of the genetic abnormalities can be fully studied. Single fetal cells allow more detailed and direct analysis of single cell genome by routine quantitative fluorescence polymerized chain reaction (QF-PCR), fluorescence in-situ hybridization (FISH), and even karyotyping on cultured fetal cells (Figure 1). The recent advances in single cell isolation by high performance multi-colour cell sorter and single cell genomic analysis by high resolution array comparative genomic hybridization (aCGH) and high throughput next generation sequencing (NGS) further promote single fetal cell NIPD in near future [13]. Fetal cells are rare in maternal circulation, this could increase the challenge to isolate the fetal cells from maternal blood. The developed isolation and enrichment protocols vary among investigators and even not reflect the target fetal cells [14]. How to improve the sample automation and avoid molecular contamination will be another major challenge. By overcoming these technical challenges, the fetal cells NIPG methods should provide new and promising alternative opportunities and application platforms for useful and reliable prenatal diagnosis.

REFERENCES