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Review Article

Research Progress in Isolation and Enrichment of Fetal Cells from Maternal Blood

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Prenatal diagnosis is an important means of early diagnosis of genetic diseases, which can effectively reduce the risk of birth defects. Free fetal cells, as a carrier of intact fetal genetic material, provide hope for the development of high-sensitivity and high-accuracy prenatal diagnosis technology. However, the number of fetal cells is small and it is difficult to apply clinically. In recent years, noninvasive prenatal diagnosis (NIPD) technology for fetal genetic material in maternal peripheral blood has developed rapidly, which makes it possible to diagnose genetic diseases by fetal cells in maternal peripheral blood. This article reviewed the current status of fetal cell separation and enrichment technology and its application in noninvasive prenatal diagnosis technology.

1. Introduction

Prenatal diagnosis refers to the detection and diagnosis of an embryo or fetal development or disease before birth. The target population can be divided into the following: advanced maternal age (≥35 years), chromosomal abnormalities of previous pregnancies or spouses, presence of genetic disorders in the family, congenital abnormalities, mental retardation, and increased risk of diagnostic testing. Prenatal diagnosis is mainly divided into invasive prenatal diagnosis and noninvasive prenatal diagnosis (NIPD). Invasive prenatal diagnosis uses interventional means to obtain fetal genetic material for analysis, including amniocentesis, umbilical cord puncture, and transcervical villus biopsy (TC-CVS) and transabdominal villus biopsy (TA-CVS), which can be performed in early pregnancy, but they have a 0.3–0.1% risk of procedural miscarriage [1, 2]. Some of these technologies have come into play late, while others are limited to a few specific abnormalities and disease detection, and they have limitations in terms of timeliness, sensitivity, and scope of detection. Therefore, noninvasive prenatal diagnosis is becoming a hot topic. Compared with invasive prenatal diagnosis, NIPD has no risk of abortion, infection, and other risks, is simple to operate, and is more easily accepted by pregnant women who need further testing.

NIPD mainly includes diagnosis technology based on plasma cell-free fetal DNA (cffDNA) and prenatal diagnosis technology by obtaining fetal cells in maternal peripheral blood and rare fetal cells in exfoliated cells. DNA fragment diagnosis technology based on placental cells circulating in maternal blood has been widely accepted in clinic for detecting common chromosomal aneuploidy [3–5]. Although fetal cffDNA is abundant and readily available in maternal plasma, prenatal screening based on cffDNA has

been used to diagnose trisomy (13, 18, 21) and other genetic diseases (e.g., thalassemia). However, cffDNA-based diagnosis has some disadvantages: (1) due to cffDNA fragmentation, it is difficult to diagnose chromosomal mosaicism, duplication, deletion, and other abnormalities; (2) cffDNA-based diagnosis requires deep sequencing with high cost and low sensitivity [6–8]. Compared with the former, fetal cells contain complete cell structure and a full set of genomic information, and, with the progress of single-cell genomic detection technology, research results show that fetal cells have been able to analyze single-cell DNA accurately and specifically [9–11]. If these fetal cells were successfully isolated and their genomic DNA was amplified on a genome-wide scale, many types of genetic changes, including chromosome reversals and translocations, could be clearly detected and, in principle, repeated amplification could be done. Therefore, this cell-based NIPD is considered as a potential diagnostic test [12, 13]. Circulating fetal cells have been reported to be rare cells shed from trophoblast or umbilical cord blood into the maternal peripheral blood. Usually 1 mL of peripheral blood contains 1×109 red blood cells and 1×106 white blood cells but may contain only 1–10 fetal cells. Although fetal cells contain complete fetal genome information, due to the small number of these cells, effective isolation, enrichment, and identification are the primary premise of using fetal cells for related genetic analysis. This paper reviews the research progress of fetal DNA noninvasive prenatal testing based on fetal cells and prospects the future development of this field.

2. Classification of Fetal Cells in Maternal Peripheral Blood

Fetal cells in the peripheral blood of pregnant women are mainly divided into four categories: fetal nucleated red blood cells (FNRBC), trophoblasts, leukomonocyte, and granulocyte may form the cells.

Studies have shown that fetal cells exist in maternal peripheral blood during pregnancy; compared with maternal peripheral blood cells and epithelial cells, fetal cells in maternal peripheral blood content are very small; usually 6–8 fetal cells may only exist in 1 ml of maternal blood. The number of fetal cells in maternal blood at a given time is reproducible and can therefore be assessed by cytogenetic methods [14]. DNA has been successfully isolated from fetal cells circulating in the blood of pregnant women and can be used to diagnose fetal sex [3]. However, fetal lymphocytes and granulocytes may develop maternal tolerance to the fetus, or to maternal autoimmune diseases, and it can continue for many years [15]. This persistence makes circulating fetal lymphocytes and granulocytes unsuitable for NIPD, as their presence in subsequent pregnancies may influence test results. In contrast, FNRBC and trophoblast cells were cleared from the maternal circulation rapidly after delivery and were not detected after ≥8 weeks [16]. Therefore, at present, trophoblast cells and fetal nucleated red cells are mainly studied as fetal cells in prenatal diagnosis. However, despite cell-based enrichment methods, fetal cells are difficult to grow without contamination because only a very small number of them are present in maternal blood [3].

2.1. Fetal Nucleated Red Blood Cells. Among fetal cells in the maternal peripheral blood, fetal nucleated red blood cells are the most ideal cells for prenatal screening. Because FNRBC have complete fetal genetic information, the accuracy of identification in maternal blood cell populations is high, and the survival time and life cycle of FNRBC are short. After delivery, they will disappear completely in the maternal peripheral blood within 3 months. Prenatal examination Time will not be affected by past pregnancies [10]. However, its amount in the maternal blood circulation is very small, which affects the direct use of it for prenatal diagnosis. After pregnancy, the placenta becomes the communication bridge between the mother and the fetus. Similarly, maternal and fetal cells can exchange through the placenta, and the fetal cells pass through or fall off the villi and sinus space and enter the maternal circulation. The number of fetal nucleated red blood cells in the maternal peripheral blood can be affected by many factors, such as the number of red blood cells produced by the fetus itself, the integrity of the placental structure, and the immune status between the mother and the fetus. In addition, different cell capture and sorting methods can draw different conclusions because of their different sensitivity and specificity. Studies at home and abroad have shown that, under pathological pregnancy conditions with abnormal maternal placenta, such as gestational hypertension and gestational diabetes, the number of fetal nucleated red blood cells in maternal blood is significantly increased [17]. FNRBC can be seen in the peripheral blood of pregnant women in early pregnancy, with short survival time, significant morphological characteristics, and certain cell surface markers [3]. At present, the identifiable markers of fetal nucleated red blood cells include FNRBCs surface or intracellular specific antigens, such as CD71, GPA, globin, CD36, HLA-G, and EPO-R. The cells can be labeled and screened by these positive markers. Zhang et al. [18] used a microfluidic chip coated with anti-CD71 antibodies to identify 5–35 FNRBCs per 2 ml of maternal blood starting from 7 weeks of pregnancy, and SRY-PCR confirmed the fetal origin. However, studies have shown that these positive antibodies are not highly specific, leading to large false positives [14, 19]. It shows that, for rare fetal cells, the loss of fetal cells can be derived from the positive antibody enrichment method. Therefore, in subsequent research, a combination of positive antibody labeling and negative antibody labeling was used, and the combination of cell surface labeling and intracellular labeling was used to improve the capture efficiency.

2.2. Trophoblasts. Circulating trophoblast (CTB) cells are a type of placental-derived cells. Because of their large size, special morphology, and easy identification, they are the easiest cells to separate in theory. Compared with cell-free DNA, an important advantage of trophoblasts is that they carry the entire fetal genome without maternal DNA contamination. The first cells found in the mother's body are trophoblast cells, which are different from fetal lymphocytes and fetal bone marrow cells. They will not stay in the mother's body for many years after delivery, which will affect

the test results. Therefore, they have a unique shape and are closely related to the mother's body, and they are considered to have the biggest potential to isolate fetal cells. However, there are still some problems in the application of trophoblasts: ① the best diagnosis time of trophoblasts is early pregnancy, but only a few trophoblasts exist in the peripheral blood circulation of pregnant women. ② Because trophoblasts are large in size, they are easy to stay in the lung tissue, resulting in a small amount of maternal peripheral blood. ③ The specificity of trophoblast monoclonal antibody HLA-G and CD105 is not high. ④ Because trophoblasts come from placenta, polynuclear characteristics and chimeric karyotype of aggregated trophoblasts will interfere with the analysis of genetic results. Chung-Er Huang et al. [20] used a specific antibody, EpCAM, to connect to a silicon-based nanostructured microfluidic chip to immunoadsorb the trophoblasts in the maternal peripheral blood and then used cytokeratin-7 (+)/HLA-G(+)/DAPI(+) immunofluorescence staining to identify fetal trophoblasts, HSH, aCGH, STR analysis, and NGS technology for prenatal diagnosis of chromosomal diseases. Studies have shown that rare trophoblast cells can be used to diagnose 47, XXY, T18, and T13 syndromes [10]. To sum up, the fetal cells in the maternal peripheral blood contain the complete genetic information of the fetus and are one of the ways to diagnose single-gene diseases. However, due to their small number and difficulty in isolation, clinical transformation is limited.

2.3. Lymphocytes. Since the discovery of the karyotype of the male fetal lymphocytes in the peripheral blood of pregnant women in 1969, a scientific research team has successfully isolated the fetal lymphocytes from the maternal blood, but the fetal lymphocytes enter the maternal blood circulation relatively late. Due to the slow circulation of fetal lymphocytes into the mother's blood, the number of early pregnancy in the mother's blood is small, not in the prime of prenatal diagnosis. Secondly, lymphocytes still exist in the maternal peripheral blood for many years after delivery, which affects the prenatal diagnosis results of the second pregnancy [3]. In addition, fetal lymphocytes lack specific monoclonal antibodies. Therefore, fetal lymphocytes are difficult to use for prenatal diagnosis.

2.4. Granulocyte. The results of fetal granulocyte research are very rare. Only one team successfully isolated granulocytes from the mother's peripheral blood. The team used Ficoll density gradient centrifugation and flow cytometry to separate cells from the peripheral blood of a female who had not given birth to a male baby and used FISH to identify Y signal. This report may be due to a technical error or male cells are derived from a previous pregnancy history. Fetal granulocytes also account for 0.13%–0.26% of maternal peripheral blood mononuclear cells, but there is no effective data showing that fetal granulocytes can be used for fetal cell separation and noninvasive prenatal diagnosis.

3. Main Methods of Separation and Enrichment of Fetal Cells in Pregnant Women's Peripheral Blood

Because fetal cells contain 1 fetal cell in about 105 to 109 maternal cells in the peripheral blood of the mother and their number is very small, it must be separated and enriched before it can be used for noninvasive prenatal testing. The commonly used methods so far are density gradient centrifugation (DGC), filtration on chip, magnetic activated cell sorting (MACS), fluorescence activated cell sorting (FACS), microscope operation method, and so forth. The above methods have different fetal cell recovery rates and extraction purity. Therefore, a combination of different techniques and methods is usually used to improve sample purity and enrichment efficiency. Each enrichment and separation method has its own advantages and disadvantages.

3.1. Density Gradient Centrifugation (DGC). The density gradient centrifugation method uses the density difference between the fetal cells in the maternal blood and other cells in the peripheral blood to mix and centrifuge the Ficoll of the appropriate density with the peripheral blood and place the target cell layer in a specific density zone to separate the target cells. Studies have proved the role of this method in enriching nucleated cells and removing maternal red blood cells. According to the density of the medium used, it can be divided into single-density gradient centrifugation, double-density gradient centrifugation, and discontinuous density gradient centrifugation. Studies have shown that fetal cells can be separated from maternal blood by double-density gradient centrifugation [21]. In 2018, Feng et al. used density gradient centrifugation to initially separate fetal nucleated red blood cells and then captured them on a microfluidic chip coated with CD147 antibody and finally obtained 22–56 cells per milliliter of peripheral blood [22]. Domestic scholars Xu et al. [23] used density gradient centrifugation to concentrate peripheral blood mononuclear cells (PBMCs) from whole blood for the first time, greatly increasing the number of nucleated cells.

DGC is relatively simple in operation, short in time, and low in cost, but it is usually used as the first step of FNRBC enrichment, and then the target cells are further purified, because the number of enriched fetal cells is small and the purity is low.

3.2. Fluorescence Activated Cell Sorting (FACS). FACS is a technology to enrich and separate the target cell population by using the surface antigen of the target cell which specifically binds to fluorescent antibody. In 2017, Chen et al. [19] developed a double negative selection (DNS) method to isolate fetal cells from maternal peripheral blood. The method includes first using red blood cell lysate to remove red blood cells and then using magnetic beads to couple monoclonal antibodies against leukocyte surface antigen CD45, using the principle of antigen-antibody specific

binding to remove leukocytes, and then using FACS to further remove nontarget cells to obtain a suspension of a large number of target cells, and finally single cells are selected by the morphology of the fetal cells. In 2021, a study took male pregnancy cases as the research object, and FNRBC was isolated from the blood of pregnant women through FACS [24]. In order to isolate fetal cells from endocervical specimens and try to identify possible abnormalities, Erkan et al. [25]used human leukocyte antigen (HLA) G233 and placental alkaline phosphatase (PLAP) antibodies to separate fetal cells from cervical intima specimens by fluorescence activated cell sorting (FACS) and magnetic activated cell sorting (MACS). The results showed that the percentages of HLA-G233 and placental alkaline phosphatase (PLAP) positive cells were 4.55% and 84.59%, respectively. The positive rate of the two markers was 14.75%.

FACS enrichment is reliable, and the specificity of its antibody determines the purity of sorted cells. However, the cost of experimental reagents and equipment is high, the application is difficult and takes a long time, and it requires professional operations.

3.3. Microfluidic Chip Filtration Method. Microfluidic chip filtration method is to separate target cells by using chip microchannels and the specific size and shape of cells themselves. In 2015, Han et al. [26] reported that a microfluidic chip was used to separate FNRBC from maternal blood by a two-step cascade enrichment method. In 2017, Chinese scholar Zhao et al. [27] developed a biocompatible nanostructured microfluidic chip, which can not only separate FNRBC from maternal peripheral blood very effectively but also realize the in situ bioanalysis of FNRBC on the chip. Foreign scholars Benjamin Thierry et al. [28] published an inertial-based microfluidic chip technology to separate trophoblast cells in 2018. By removing red blood cells, the cells were introduced into the chip, and the target cells were separated according to the different sizes of white blood cells and trophoblast cells and the inertia received in the chip. Finally, the target cells were identified by fluorescence in situ hybridization (FISH) and gene sequencing, and the results were analyzed. The recovery rate can reach 79%.

The microfluidic chip method has high sensitivity, but, due to the high technical and laboratory requirements and expensive equipment, there may be a greater risk of sample contamination or loss. Therefore, the technology needs to be further optimized, and the fetal cell sorting effect needs further research.

3.4. Magnetic Activated Cell Sorting (MACS). MACS uses the antibody labeled by magnetic beads to specifically bind to the target cell antigen and uses the strong adsorption of magnetic beads such as attaching magnetic frame to separate the target cells, which is relatively cheaper than FACS and is widely used in the study of fetal nucleated red cells sorting. In 2019, Liesbeth Vossaert et al. used the MACS method to enrich trophoblast cells and finally enriched to 5.38 cells per 28.5 ml in maternal peripheral blood [29]. Foreign scholar Dragos Nemescu et al. [21] first separated fetal cells from maternal blood by double-density gradient centrifugation and then selected magnetic cells according to the paramagnetism of NRBC hemoglobin, converted into methemoglobin, or used anti-CD71 monoclonal antibody for positive magnetization activated cell sorting enrichment. Finally, the cells are identified by fluorescence in situ hybridization with specific chromosome X and Y probes.

MACS sorting method has the advantages of short time and relatively low cost of separating multiple samples at the same time, and the cells remain active after separation. The disadvantage is that, like FACS, the separation purity of MACS depends on the specificity of antibody, and it is difficult to avoid mother cell contamination, so it should be used in combination with other methods.

3.5. Microscope Operation Method. Microscope operation method is to accurately separate the target cells according to the characteristics of cells to be separated. Under the operation of platform and system, the whole operation process can be clearly seen, thus avoiding the loss of cells and the mixing of nontarget cells. Katarina Ravn et al. [30] collected blood from 13 pregnant women, used MACS method to separate and enrich circulating fetal trophoblast cells, stained with anti-cytokeratin antibody, and identified target cells using MetaSystems fluorescence microscope scanner. The advantage of this method is that it can identify and obtain a single target cell from the morphology, and the cell purity is high, which can help us distinguish the fetal origin and maternal origin of fetal cells, but it is rare in the blood circulation of pregnant women. In the separation of target cells, due to the existence of a large number of nontarget cells, the micromanipulation separation method takes too long and the workload is huge, which is not conducive to the preservation and separation of rare samples. At the same time, it also has the disadvantages of expensive operating equipment and high requirements for operating technology.

3.6. Method Based on Nanometer Material and Microsphere Material. The method based on nanomaterials and microsphere materials is to add microfluidic sorting platform or microsphere sorting platform to the original antibody capture, which significantly improves the antibody capture efficiency. In 2017, Tseng et al. [10] used PLGA nanosubstrate structure combined with a herringbone microfluidic chip to realize the separation and capture of cTBs. Domestic scholar Lin Cheng et al. [31] used density gradient centrifugation for preliminary separation in 2019 and then captured FNRBC with SiO_2 microspheres coated with CD147 antibody and obtained 42–93 FNRBC/ml in peripheral blood. Wei et al. [32] combined with nucleated red blood cells by using microspheres coated with CD147 and separated by high-density Percoll centrifugation and finally enriched nucleated red blood cells with an efficiency of 84% and a purity of 80%. In summary, on the basis and with application of a large number of experiments, we found that a single method is difficult to achieve stable and efficient fetal

cell separation. It requires multiple conditions for simultaneous separation, and the efficiency, time, and cost are as close as possible to the clinical requirements.

4. Identification and Application of Fetal Cells of Pregnant Women

The fetal cells enriched and purified by the above methods are not all fetal sources. Studies have shown that about 22–50% of nucleated red blood cells enriched and purified from pregnant women's peripheral blood come from mothers [33]. Therefore, purified fetal cells from pregnant women must be identified as fetal-derived or maternally derived before they can be used for prenatal testing. At present, the commonly used methods are as follows.

4.1. Fluorescence In Situ Hybridization (FISH). Fluorescence in situ hybridization uses specific sites of Y chromosome to design fluorescent probes, and, after hybridization, fluorescent staining can identify male fetal cells. FISH technology, which can be used as a cytogenetic method in maternal blood, is one of them. It can directly screen out abnormal chromosome cells. In 2020, foreign scholars [21] collected the peripheral blood of 27 pregnant women and enriched and separated fetal cells through DGC and MACS. The FISH analysis found at least one XY cell in 81.5% and 61.5% of cases, respectively, for paramagnetic and anti-CD71 selection. Some studies have used FNRBC specific antibodies (anti-CD147) to modify gelatin-coated silica beads to capture target cells in blood samples and then purify them. Then the released cells are analyzed by real-time PCR to verify their fetal origin, and FISH is used to detect fetal chromosomal abnormalities. The final test showed that 2 pregnant women with male fetuses were confirmed; 4 fetuses with 21-tris syndrome and 3 fetuses with trisomy 13 were confirmed [32]. He et al. [27] used an immunoaffinity chip to separate FNRBC from maternal blood and confirmed the fetal origin of the separated cells by FISH analysis.

FISH identification is short in time, low in price, and high in accuracy, but the disadvantage is that it cannot be used for quantitative analysis of cells, and the identified cells cannot be used for other detection methods, which is a loss of cell quantity.

4.2. Polymerase Chain reaction (PCR). PCR refers to the technique of obtaining exponentially amplified DNA sequences by repeating the DNA replication cycle, each cycle including DNA denaturation, primer annealing, primer elongation, and other processes. It can amplify the specific sequence of the entire fetal genome, obtain enough DNA for analysis, and use a minimum number of cells and quantitative analysis to identify the source of the cells. Yang et al. [34] used multiplex PCR, target capture, and next-generation sequencing to perform noninvasive prenatal diagnosis of thalassemia. Guissart et al. [35] used a universal detection method for indirect diagnosis of cystic fibrosis (CF) based on fluorescence multiplex PCR and large and small fragment analysis. The results showed that noninvasive prenatal diagnosis from maternal peripheral blood was successfully applied 30 couples. The human leukocyte antigen sequence is specifically expressed by fetal cells, not limited to male fetal cells, and can also be identified and quantified by PCR technology. At present, the sensitivity of this type of technology has been improved, and target gene amplification can be achieved in single cells.

4.3. Whole Genome Amplification (WGA). Single-cell whole-genome sequencing technology is a technology to amplify and sequence the entire genome at the single-cell level. The main principle is to amplify a small amount of whole-genome DNA in a single cell. Then, after obtaining a high-coverage whole genome, high-throughput sequencing is performed. The prerequisite for obtaining accurate and comprehensive sequencing results is high-coverage, high-fidelity whole-genome amplification products. At present, there are three main methods for the more commonly used WGA : degenerate oligonucleotide PCR technology, multiple displacement amplification, and so forth. For cells fixed with soluble polytetrafluoroethylene PFA, various WGA methods need to be optimized. In 2016, the whole-genome approach was used to explore NIPD whole-genome sequencing for single-gene genetic diseases, combined with a series of bioinformatics screening, to increase the positive predictive value of new fetal mutation detection to 74% [36]. Scholar Weymaere et al. [37] discussed the effectiveness and evidential value of STR and SNP genotyping methods for 24 single cells after WGA in three families, using formaldehyde-fixed cells and unfixed cells in the offspring-parent combination. The results showed that the two genotyping methods could be used in all test conditions and scenarios, and the sensitivity and specificity are 100%, and the evidence values for fixed cells and nonfixed cells are similar. In addition, sequence-based SNP genotyping has higher evidential value than length-based STR genotyping after WGA, which cannot be observed using a large number of high-quality progenitor DNA samples.

4.4. High-Throughput Sequencing Technology (NGS). High-throughput sequencing technology can comprehensively analyze the genome and transcriptome of a species. Compared with traditional PCR or FISH, NGS can provide high-throughput and basic-level genetic analysis information more quickly, and NGS can detect and analyze multiple gene loci at the same time, which is the gold standard for maternal and fetal cell recognition. It does not need multiple restriction endonuclease digestion reactions, and the accuracy rate is higher than that of traditional methods, reaching 100%, which is the direction of cell recognition in the future. NGS still has some disadvantages, such as high cost and inconvenient separation of target cells. Hua et al. [38] found in 2015 that the whole-genome sequencing (WGS) method can successfully detect aneuploidy diseases in individual FNRBC. With the increasing optimization of technology, those diseases caused by insertion/deletion and point mutation are expected to be confirmed by fetal cells in maternal blood. Yu et al. [39] used NGS to detect fetal aneuploidy, and

NIPD was used to detect aneuploidy in 26 fetuses, of which 18 fetal aneuploidies occurred in only one fetus of twins. The aneuploidy rates of double-chorionic twins were 1.3% and 0.5% of single-chorionic twins. The incidence of aneuploidy was 1.2% in the spontaneous pregnancy group and 1.1% in the assisted reproductive technology group. Huada Gene Research Group, for the first time, confirmed that the fetal cells separated by double negative selection combined with deep sequencing (used for screening single-gene diseases) had high-coverage rate of WGS (86.8%) and allele loss rate of 24.90% [19]. In 2021, the scholar Noriko et al. [24] have found that FNRCs in the maternal circulation can be selectively separated by single-cell sorting, and their fetal origin can be confirmed by real-time PCR and WGS.

Fetal cells in the peripheral blood of pregnant women can be used as a source of genetic material for prenatal testing after identification, and corresponding molecular biology techniques can be used to detect diseases. At present, FNRBC has been applied to noninvasive prenatal diagnosis, detecting fetal-related phenotype and diseases such as fetal sex, the blood type, chromosome abnormality, and HLA polymorphism. Some scholars [31] carried out prenatal testing of the fetal ABO blood type by capturing FNRBC and testing the obtained samples. It was shown that the results of testing the fetal blood type were consistent with the results after birth. In 2018, Zhang et al. [18] used fetal nucleated red blood cells to conduct gender identification. Experiments showed that the gender of 20 samples of fetuses was consistent with the detection results of free DNA. At present, FNRBC can be labeled and screened by corresponding markers, such as CD36, HLA-G, globin, and CD71.

In recent years, due to the discovery of new antigens on the surface of trophoblasts and the introduction of new enrichment methods, the application of trophoblasts in prenatal diagnosis has attracted people's attention. A study in 2016 pointed out that the multisite STR and SNP sequencing analysis of the enriched trophoblast cells found that their genotypes are completely consistent with the placental genotypes [40]. Vossaert et al. showed in 2018 that circulating trophoblast cells can be separated by single cells for the detection of chromosome microdeletion, with a resolution of up to 1 to 2 Mb [41].

For fetal lymphocytes and granulocytes, fetal lymphocytes are similar to maternal lymphocytes in immunology, and it is not easy to find fetal specific antibodies, so it is not the best choice for prenatal diagnosis. At present, there is no report of successful isolation of fetal granulocytes, so it is rarely used in prenatal diagnosis.

With the development of technology, fetal cells in maternal peripheral blood will play an important role in monogenic genetic diseases. Besides, these cells can also be used to understand fetal ABO type [31]. It has a certain significance for the diagnosis of neonatal hemolysis. NGS can be used to identify the fetal genotype, which is also of guiding significance to the nutrition absorption during pregnancy and prenatal and postnatal care.

5. Problems and Prospects

In conclusion, maternal peripheral blood fetal cells have a wide range of applications and development prospects in prenatal diagnosis and prenatal screening, and technical optimization and clinical data demonstration are still needed to evaluate whether it can truly surpass the existing NIPT detection and become a real technical method for clinical application. At present, the enrichment and detection methods of fetal cells in maternal peripheral blood are not perfect. Therefore, we need to further search for more effective and more sensitive identification methods. In addition, a unified quality evaluation standard should be established for the various enrichment and detection methods developed, which is more conducive to promoting the clinical transformation of circulating fetal cells. It is believed that, with the continuous development and optimization of science and technology, its application prospect is more and more broad. The detection methods of fetal cells in peripheral blood of pregnant women will get more choices and be widely used in prenatal diagnosis.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

Ying Tang and Qiaojin Tang are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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