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CELL-FREE DNA

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Abstract: Cell-free DNA, or cfDNA, has been increasingly recognized as an important biomarker in several areas of medicine. cfDNA circulates in the blood and other body fluids, originating from the apoptosis and necrosis of cells in the body. cfDNA analysis has shown promise in the early diagnosis of disease, in the follow-up of treatment and in the detection of cancer. Recently, technological advances have allowed the analysis of cfDNA with high sensitivity and specificity. The use of next generation sequencing techniques allows the detection of specific genetic mutations associated with different categories of cancer, allowing the development of more accurate and effective tests for the diagnosis and treatment of cancer (Bratulic, S. 2022). Furthermore, cfDNA analysis has been applied in other areas of medicine, such as detecting infectious diseases and monitoring organ transplants. cfDNA analysis also has the potential to be used as a non-invasive biomarker for the prenatal diagnosis of chromosomal abnormalities.
Keywords: Cell Free DNA; Early Cancer Detection; Prenatal cfDNA Screening

INTRODUCTION

Cell-free DNA, also known as cfDNA, is a DNA molecule that can be found in an individual's bloodstream. cfDNA analysis is an innovative diagnostic technique that has the potential to revolutionize the way diagnostic medicine is performed. Its detection has been used as a promising tool, especially in the field of oncology. One of the most promising applications of cfDNA analysis is in cancer detection.

Studies show that circulating cfDNA contains specific mutations that can be used to detect cancer non-invasively, often before symptoms appear. This may allow for earlier diagnosis and therefore more effective treatment and better patient outcomes. As a non-invasive technique, cfDNA analysis offers a number of advantages over conventional approaches such as biopsy and fine needle aspiration (Vidula, et al, 2021). Unlike these techniques, cfDNA analysis is less painful and stressful for the patient and can be used to detect a variety of diseases and conditions, as in prenatal diagnosis, allowing the detection of genetic abnormalities, autoimmune and infectious diseases without the need for invasive procedures such as amniocentesis. This can significantly reduce the risk of complications for both the mother and the fetus, as well as providing valuable information to guide prenatal care. cfDNA analysis can be performed using advanced genomic sequencing techniques and other molecular analysis approaches.

METHODS

The methodology chosen for research and development was bibliographical research, which involves deepening the proposed objective content. The research was carried out through searches in articles, and academic sites, such as Google Scholar and Scielo.

DISCUSSION

Methods around cfDNA are non-invasive. Cell-free DNA extraction can be performed from different types of biological samples such as blood, saliva, urine, blood plasma or amniotic fluid. The extraction protocol will depend on the type of sample and the experimental objective. The sample must be collected and stored correctly, following specific instructions for each type of sample. (CHEN, 2021)

It is important to avoid contamination from other samples and to ensure the integrity of the DNA. After collection, the sample must be processed to separate the cells. This can be done by centrifugation, filtration or other methods. Cell-free DNA can be extracted

using a few different methods, such as:

- Extraction with phenol-chloroform, this method is based on the separation of DNA from proteins and other contaminants. using the organic phase of phenol-chloroform. It is widely used in research laboratories and is suitable for samples of blood and other body fluids;
- Silica gel column extraction, this method uses a silica gel column to purify the cfDNA. This is a fast and efficient method, suitable for samples containing low concentrations of cfDNA
- Salting-out extraction, this method uses salt precipitation of DNA to remove proteins and other contaminants. This is an easy and cost-effective method. but may result in low concentrations of cfDNA;
- Ultrasonic Extraction, this method uses ultrasound to destroy cells and release cfDNA. It is suitable for both solid and liquid tissue samples.

Analysis of cfDNA can provide information about the effectiveness of chemotherapy, for example, low levels of cfDNA during chemotherapy can indicate the effectiveness of the treatment. Your analysis can also help detect cancer recurrence after treatment. Detection of specific tumor-associated mutations in high levels of cfDNA may indicate that the cancer is recurrent, even if it is not yet detectable by imaging tests. Recognizing cfDNA can help personalize cancer treatment by identifying specific mutations that respond best to certain treatments.

Overall, cfDNA analysis aids in clinical decision-making during chemotherapy and allows treatment to be adjusted to improve effectiveness and reduce side effects.

In recent years, there has been a significant focus in clinical research on the use of donor-derived cfDNA (dd-cfDNA) in solid organ transplants. While most studies have focused on kidney transplants, research is also being done on heart, lung, pancreas, and liver transplants.

It became clear that dd-cfDNA is not a specific marker for transplanted organ rejection, but rather an indicator of severe injury, with organ rejection being the most common cause of this injury. Combining dd-cfDNA with new urine or blood biomarkers may increase sensitivity in the diagnosis of rejection.

In solid organ transplantation, genetic differences play a key role. With the exception of donor-recipient identical twins, this procedure inserts a unique genome into the recipient, which theoretically facilitates the detection of circulating donor DNA through a minimally invasive blood draw. Furthermore, this biomarker may reflect graft integrity at low levels and cell death at high levels. Special focus has been placed on the dynamics of this DNA during rejection, as this aspect of transplantation currently requires invasive biopsies. This is particularly relevant in heart transplantation (HT), where routine biopsies are controversial and generally only performed when clinically indicated. A liquid biopsy can represent a revolutionary approach in this scenario.

Although current data do not allow a definitive conclusion, ongoing studies may reveal the usefulness of continuous surveillance in the management of solid organ transplants. Monitoring dd-cfDNA levels over time may offer opportunities for early interventions before irreversible graft damage occurs.

It is also possible to prenatally diagnose chromosomal abnormalities using cell-free DNA present in maternal blood. cfDNA is released into the maternal bloodstream by the placenta and contains fragments of fetal DNA that can be analyzed to detect chromosomal abnormalities such as Down syndrome (trisomy 21), Edwards syndrome (trisomy

18) and Down's syndrome (trisomy 18). Patau syndrome (chromosome 13 trisomy), among others. (La Coursiere, et al, 2021)

cfDNA analysis can be performed from the 10th week of gestation and is considered a non-invasive option compared to other techniques such as amniocentesis or chorionic villus sampling, which carry a small risk of complications for the mother and fetus.

However, it is important to remember that the diagnosis using cfDNA is not 100% reliable and may require complementary tests to confirm or rule out the detected anomalies.

CONCLUSION

In conclusion, cfDNA has been used promisingly in several fields of medicine. Its analysis offers advantages, such as being non-invasive and allowing the detection of specific mutations associated with different medical conditions. With technological advances, cfDNA is expected to be increasingly used as a valuable biomarker to improve patient care and clinical outcomes. It is important to invest in research to improve the accuracy and reliability of cfDNA analysis, in addition to exploring new applications for this innovative technique.

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