Review Article

Advances in the medical research and clinical applications on the plasma DNA

Shuye Wang[1], Yuanyuan Chen[1], Zhanhe Wu[2]

[1]Department of Hematology, The first Affiliated Hospital of Harbin Medical University, Harbin 150001, China; [2]Western Sydney Genomic Diagnostics, Western Sydney Genetics Program, The Children's Hospital at Westmead, NSW, Australia

Correspondence to: Shu-Ye Wang. Department of Hematology, the First Affiliated Hospital of Harbin Medical University, Harbin 150001, China. Email: wsywangshuye@126.com.

Abstract: Plasma DNA has had a strong impact and influence on basic medical research and clinical practice since the discovery of low levels of plasma DNA in healthy individuals under different physiological conditions. Although the source of circulating DNA still requires further investigation, a wide range of research has also proven the value of qualitative and quantitative measurements of plasma DNA in many disease conditions. The use of plasma DNA has a biomarker is advantageous due to accessibility, reliability, reproducibility, sensitivity, specific and relatively low cost. Recently, the detection of circulating (plasma) DNA quantitative changes have been using in the studies on the tumor gene mutations and to monitor disease progressing and to predict the disease prognosis. Such technique also has been using other many different fields, particularly in prenatal diagnosis, for which plasma DNA testing is preferable due to non-invasiveness. This article reviews the research progression and clinical applications of plasma DNA in the last several years.

Keywords: Plasma DNA; haematological malignancies; non-invasive tool at diagnosis

Submitted Jan 28, 2014. Accepted for publication Mar 18, 2014.
doi: 10.3978/j.issn.2224-4336.2014.03.07
View this article at: http://www.thetp.org/article/view/3550/4413

Introduction

Circulating DNA is free nucleic acids mainly present in the peripheral blood cells, synovial fluid and other body fluids. In the early 60s, there has been suggested that there may be free nucleic acid in the blood circulation, but it was not reported until 1977 by Leon *et al*. (1), with the finding to demonstrate that tumor derived DNA exists in the plasma of the patients with tumor and that the plasma DNA level in patients with cancer is significantly higher than that in the normal individual. In recent years, with the rapid advances in molecular biological technologies, the study on circulating DNA arouses attention dramatically. A new study using real-time PCR for the quantification of circulating DNA found that elevated levels of circulating DNA in patients with tumor are associated with disease characteristics and prognosis (2). This article reviews the advances in basic medical research and the clinical application of plasma DNA.

Research progressing of plasma DNA

Structure characteristics

Circulating cell-free DNA (Cf-DNA) refers to a molecule of DNA in plasma with double helix structure and a size between 500-30,000 bp nucleotides. Research showed that circulating DNA consists of admixtures of double stranded and single stranded DNA. There are two existence forms: the free DNA and DNA-protein complexes, with the former existing in plasma in the free form, the latter combining with proteins into complexes or, in blood, attaching onto the surface of blood cells (3). The content is very low, with concentrations at only nanograms per milliliter, often less than 10 ng/mL. The short half-life is about 10 to 15 minutes, due to mostly hepatic clearance, but also due to elimination in the kidneys.

The source of Cf-DNA is from dead cells in healthy individuals as they are mainly cleaned up by circulating phagocyte and can be nucleic or mitochondrial in origin.

Plasma levels of DNA and protein are combined in the form of ion single nucleosome or oligo nucleosome. After the cells dead, their connection point is cut off by enzyme, and they release the DNA into circulation. Single nucleosome contains an eight mer (two times the amount of H2A, H2B, H3 and H4) core particles, and spiral DNA peripheral consists of a ring extension 146 bp surrounded.

Oligosaccharides consist of single nucleosome through the DNA connector (including DNA variable length 15-100 bp). Once it is released into the blood circulation, the nucleosome can protect the structure from the degradation (3) by the enzyme. About 1.5% serum proteins have the characteristics of wrapped DNA. Albumin, IgM, IgG, Clq complex, fibronectin, lactoferrin, and lysozyme also have the certain properties to combine with DNA in order to protect the circulating DNA. In addition, plasma DNA may bind to certain structures on cell surfaces. DNA can bind to the surface of leptosomes and cell membrane in two divalent metal cation conditions, may be the phosphate and phospholipids in DNA metal combined through two metals. The cell membrane in red blood cells, white blood cells and platelets also has high affinity for circulating DNA and protein. Cell surface proteins contain positively charged amino acids which attract DNA molecules. It also facilitates the binding between the cell membrane and the extracellular nucleic acid with the apoptotic bodies, nucleosome or protein to from complexes, allowing plasma DNA to circulate for longer in the blood. The occurrence mechanism: The exact mechanism of circulating free DNA source has not yet been elucidated. There are five kinds of mechanisms for patients with tumors mainly as follows: (I) cells actively secreting or releasing DNA during DNA replication, whereby active proliferation of cancer cells result in the sustained release of newly synthesized DNA fragments into the extracellular and entering the blood circulation; (II) the apoptosis of tumor cells: apoptosis is characterized by DNA degradation. Firstly, the chromosome DNA decomposes into large fragments (50-300 bp) and then they are decomposed into a lot of nucleosomes (180-200 bp) after the electrophoresis of circulating DNA, a gradient can exhibit the characteristic of apoptotic cells, but the mechanism of apoptosis is a defensing of the proliferation of tumor cells, once after the cell apoptosis, apoptotic bodies containing DNA are cleaned quickly by macrophages nuclear and neighboring cells in situ, without causing increase in circulating DNA levels generally (4); (III) the inhibition of the enzymes responsible for degradation of DNA: the small amount of free DNA in normal human plasma is rapidly degraded, likely by DNAse I or II; but in the malignant tumor, this enzyme activity may be inhibited (e.g., by a very strong inhibitor of DNAseI or II in blood circulation). It results in the buildup of the level of DNA in plasma in patients with malignant tumors (5); (IV) circulating tumor cells in blood: some scholars put forward that the free circulating DNA is released into the bloodstream by intact tumor cells and the lysed tumor cells (6,7). However in the peripheral blood circulation there are not enough tumor cells to produce such high levels of circulated DNA as observed; (V) the necrosis of tumor cell: the high levels of plasma DNA can be found in the plasma of the patients with larger tumors or with metastatic carcinoma, indicating that tumor cells necrosis may release genetic material in cells into the circulation.

Studies have reported (8) that the fetal DNA in maternal circulation is due to placental apoptosis. Increased DNA levels are associated with abnormal development of the fetus, as observed in preeclampsia, intrauterine growth restriction, Down's syndrome and placenta accrete. Research has shown that healthy individuals have less circulating DNA, and the circulating DNA level change has no relation to gender or age, except for women over 60 years old. The levels of circulating DNA are closely related to the counts of patients' white blood cell count (9). Usually the serum level of DNA is many folds greater than that in plasma, due to the release of genomic DNA into the serum during the blood coagulation. Therefore DNA in plasma can reflect the basal level of DNA more accurately. In homeostasis, active, healthy cells release less DNA or the DNA is cleared by the ideal clearance mechanism. When the balance is disturbed, the concentration of circulating DNA rapidly increases.

The views above are all probable. But the exact mechanism or mechanisms involved may depend on the disease status of the patients as well as the patient's own immune status. There are more questions to be answered.

The application significance of plasma DNA in clinic

There is a small amount of circulating DNA in the plasma

of healthy individuals. Elevated circulating levels of DNA are related to many diseases and their characteristics such as cancer, pregnancy, autoimmune disease, myocardial infarction, trauma and so on.

Application of plasma DNA on haematological malignancies

Leukemia and myeloproliferative disorders

There are only few descriptive reports on Cf-DNA in hematologic malignancies comparing to studies done in solid tumors.

In patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), Rogers *et al*. (10) studied loss of heterozygosity (LOH) deletion and X-chromosome inactivation in peripheral blood and bone marrow cells and found that of 45 cases of patients with chromosomal abnormality (5q–, –7, +8, –17, or –20) as detected in the peripheral plasma LOH, 89% MDS and 70% AML patients have the same LOH in cells. In addition, among the 16 cases treated, the LOH and cytogenetic results from those 15 patients are exactly the same when they made the detection of residual disease. Among the 16 patients, 4 patients without bone marrow involved but can be detected in LOH. Among the 26 bone marrow samples, there are 19 patients with X-chromosome inactivation, all plasma samples had X-chromosome inactivation cloning. These data showed that the plasma DNA can be used to detect tumor specific DNA. Plasma DNA of the AML and MDS patients are the same with gene variant genetics and bone marrow exists of Ras mutations, FLT internal tandem duplication, and LOH.

There was another study by following up 31 remission patients which showed that the plasma DNA level increased in 5 out of 6 (83.3%) relapsed patients, whereas no increases were found in 22 out of 25 (88.0%) non-relapsed patients. The authors concluded that the quantification of plasma DNA may be useful for evaluating therapeutic effects and monitoring relapse in AML patients (11).

Lymphoid malignancies

It is reported that the higher DNA concentrations with p53 mutation from patients with non-Hodgkin lymphoma (NHL) may hold promises for the use of tumor-derived CFDNA as a tool for a non-invasive, low-cost early detection of NHL, its diagnosis and follow up of disease progression (12).

Correlations between plasma DNA and the clinical features of lymphoma

In the lymphatic system tumor, the levels of plasma DNA vary with the characteristics of lymphoma histology. Hohaus *et al*. (2) uses real time quantitative PCR to analyze the relationship between the level of plasma DNA and features of the lymphoma .They found that in diffusing large B cell lymphoma(DLBCL), mantle cell lymphoma (MCL) and Hodgkin's lymphoma (HL), the levels of plasma DNA were significantly higher and were further related to a series of clinical parameters, such as advanced age (>60 years), advanced disease (stage III/IV), B symptoms, elevated lactate dehydrogenase, and the nodular sclerosis or lymphoma blood circulation DNA accompanied by necrosis obviously increased significantly. These observations suggest that plasma DNA levels reflect not only tumor burden, but also the disease proliferation activity.

The plasma level of DNA lymphoma also can reflect the progression of the disease, by observing the main survival end point, namely the progression of the disease, the first-line treatment which is not complete remission, and the relapse or death. In 52 cases of DLBCL and HL followed up for two years, they found that the end points of lives of DLBCL and HL were 69% [95% confidence interval (CI): 47-84%] and 81% (95% CI: 65-91%) respectively, but blood circulation DNA increased in DLBCL and the end of the probability of HL patients in two years survival rates are 40% (95% CI: 9-71%) and 62% (95% CI: 35-81%) respectively, indicating that the quantitative detection of DLBCL and DNA levels in plasma of patients with HL may be an important biomarker to predict the disease prognosis as an important independent prognostic factors (2). Another study (13) reported that, among 14 cases, the detection of primary AIDS lymphoma patients plasma DNA can be carried out using multiple immunoglobulin primer, with seven cases of plasma immunoglobulin gene rearrangement persisting after chemotherapy, highlighting the risk of treatment failure in standardised chemotherapy.

Studies on plasma DNA and the detection of some common genes mutated of lymphomas

Lymphoma originates from the malignant colonial proliferation of immature lymphocytic. IGH and TCR gene rearrangements may take place during the malignant transformation process. It is reported that (14), based on

360 cases of non-Hodgkin's lymphoma (NHL) with plasma immunoglobulin gene rearrangement, plasma DNA can be detected in all 288 cases of initial treatment, refractory and relapsed lymphoma patients, while plasma DNA was not detectable for the 72 cases of complete remission after chemotherapy. The positive rate of B-NHL in patients with IGH rearrangements is 81%, the positive rate of T-NHL in patients with TCR gamma rearrangement is 44%. It shows that, the plasma tumor load in patients with lymphoma can detect circulating DNA deriving form the tumor, circulating DNA rapid cleaned after the cytotoxic therapy, it prompt soluble tumor derived DNA only can be detected in a specific stage of the disease , and it can highly predict drug resistance and prompt relapse probably. Therefore, plasmid DNA levels have the same clinical significance for the patients with NHL as the detection of the rearrangement of IGH and TCR genes from patient tissue. The measurement of the former is simpler and convenient, and much less invasive (15).

Recently (16), a new study explored the detection of rearrangement of the IgH gene region, by using PCR to detect specific DNA rearrangement fragments in the plasma of patients with NHL, and found that IgH were detected in the DNA segment reran king in all patients' plasma. This method is likely to be a more appropriate method to distinct NHL patients. Therefore, the detection of circulating IGH and TCR gene rearrangements is helpful for lymphoma diagnosis and prognosis.

Cell free EBV plasma DNA and lymphomas

Circulating EBV DNA is an exposed DNA fragment rather than an included one by a virus particle (17), and the EBV DNA fragments were relatively short, usually less than 181 bp. By studying the relationship between circulating EBV DNA and HIV infection from (18) Hodgkin lymphoma, they found that circulating EBV DNA can be seen in 91% HL and all HIV related HL. Regardless of the HIV infection status, the blood circulation of EBV DNA is higher in the advanced stages of disease, and EBV DNA in HIV negative patients was significantly higher than that of the later HL. However, effective treatment, long-term remission of EBV positive, and all the EBV of HL after chemotherapy cannot detect the viral DNA after chemotherapy. As the sensitivity and specificity of plasma EBV-DNA are high, the continuous monitoring based on levels of EBV DNA can predict the response to therapy. It can be regarded as a non-invasive biomarker of (19) EBV positive HL. NK-T cell lymphoma is a new subtype of lymphoma originated from the nose or nasal cavity, similar to EBV. The rate of EBV infection is more than 90%. Lei KI *et al*. (20) studied the expression of plasma EBV DNA before and after treatment in 18 cases of NK-T lymphoma. In 17 cases primary disease can be detected based on EBV DNA expression. The plasma EBV DNA reduced significantly or became undetectable in effectively treated patients. However, in untreated patients levels of plasma EBV DNA increased significantly. More importantly, patient survival correlated with plasma EBV DNA levels.

Studies of plasma DNA on childhood lymphomas

According to the latest reports studied on 201 children patients with lymphomas (21), the free circulating DNA levels were significantly higher than those in the control group. The plasma DNA levels correlate with age, with children aged 10 or having cfDNA levels at an average of 14 ng/mL, significantly less than those aged greater than 10 with an average of 114 ng/mL (P=0.014). It also shows that plasma DNA reflects the active proliferative disease and therefore plasma DNA may be a useful tool for the diagnosis and management of patients with lymphoma.

Studies on plasma DNA on adult lymphomas

Among the new-treated B-lymphocytes malignant tumor patients, 86% of their plasma DNA have clonal immunoglobulin rechained and rearrangement. Hohaus *et al*. (2) used real time quantitative PCR to analyse the relationship between the levels of B plasma DNA levels and clinical features and prognosis of lymphoma in 142 cases, revealing that the plasma DNA levels in lymphoma patients (with great variability among patients, mean 63.5 ng/mL, range 4.0-940.5 ng/mL) increased significantly compared with the ones in healthy individuals (mean 13.9 ng/mL, range 3.0-34.7 ng/mL).

The levels of plasma DNA varies based on the characteristics of lymphoma, plasma DNA diffusing DLBCL, MCL, and HL. It is further influenced by a series of clinical prognostic factors, such as age (>60 years), disease stage (advanced stages III/IV), the symptoms of B, and increased LDH, all of increase level of circulating reflecting disease proliferative activity. Hohaus *et al*. reported that (2) circulating DNA does not reflect only tumor burden but also dynamic states including proliferation, necrosis, and apoptosis. In particular, they found a strong association with LDH levels in all lymphoma types and with the presence of necrosis in patients with HL.

Studies suggested (22) that the use of multiple Ig primers detected plasma Ig gene rearrangement in 7 of 14 cases of AIDS associating lymphoma, which still existed after the chemotherapy. As plasma Ig gene rearrangement is a good marker specific for lymphomas, the persistence of cloning Ig gene rearrangement highlights the risk and failure of the standard of chemotherapy regimen treatment. He J *et al*. (16) explored a new way to identify the IgH gene rearrangement sequence by capture gene and detected the tumor IgH points sequence correctly in three cases of NHL patients by capturing the IgH gene region (IgCap), and this method was also used to detect the rearrangement of DNA fragments in the three cases of patients with NHL in plasma.

Greystoke *et al*. (23) determined levels of various biomarkers for lymphoma in 49 patients with HL and patients with NHL before and after chemotherapy at cycle standards. By correlating the levels of biomarkers with response to chemotherapy as assessed by 2 dimensional and 3 dimensional CT, the study showed that for all lymphoma subtypes initial nucleosome DNA levels were significantly greater in patients than those in 61 healthy control group. Nucleosome DNA is prognostic long-term disease-free survival in DLBCL. The nucleosome DNA decreased significantly during the first weeks of chemotherapy and in FL patients the decrease is indicative of long-term remission. Increased nucleosome DNA level in DLBCL patients suggests disease progression. This is further proof for the potential of cfDNA biomarkers in the assessment of the standard chemotherapy drug efficacy and toxicity, and its role towards individualized treatment in patients with lymphoma.

The main Fast Flux Test Facility (FFTF) refers to the progression of the disease, the first-line treatment is not complete remission, relapse or death, The FFTF possibility of DLBCL and HL patients was 69% and 81% separately in two years, the increase of plasma DNA levels of DLBCL patients the level of FFTF may be only 40% in two years showing that the quantitative detection of DLBCL and HL DNA in plasma can be regarded as an important indicator to prognosis. It has been reported that (19) among 74 patients with NHL (35B-NHL, 23T-NHL) and plasma IgH (FR3A/VLJH), TCR gamma (TVG/TJX) gene rearrangement, 58 patients (78.4%) who were initial treatment, refractory, recurrent can extract plasma DNA, while the 16 relieved patients were not found. In 20 cases the positive of T-NHL plasma DNA and living tissue confirmed that tumor derived DNA in plasma of patients with lymphoma.

The application of plasma DNA in the prenatal diagnostic screening

Maternal circulating DNA contains two sources of DNA from maternal and gestational fetal DNA (3-6% in total DNA).

In 1997, a study found that fetal DNA in maternal plasma provides a new chance for the non-invasive diagnosis by (24) detecting fetal DNA in maternal plasma using RT-PCR, allowing prenatal diagnosis for sex related diseases, RhD state of fetus, single gene disorders such as beta thalassemia, congenital adrenal abnormalities, and cartilage dysplasia. If the circulating fetal DNA is quantitative abnormal, it can reflect the variety of pregnancy related diseases, such as neonatal hemolytic, preeclampsia, preterm birth, fetal chromosomal abnormalities.

The large-scale clinical trials demonstrate that the use of (25) RQ-PCR detection of fetal SRY in maternal plasma and RHD had an accuracy of 100%, due to the absence of a maternal Y chromosome and hence of lack of any maternal DNA contribution in the detection of *SRY* and *RHD* genes in the plasma.

Compared to the traditional method in prenatal diagnosis (such as amniocentesis, chorionic villas biopsy), cell free DNA technology has the advantages as follows: (I) the plasma samples are easily obtained, with little trauma, which presents less danger to the mother and the fetus, and leads to higher uptake of testing by the pregnant women and their families; (II) it can be detected and used for dynamic monitoring from early pregnancy; (III) the price is cheaper relatively. Existence of circulating fetal DNA provides theoretical support and development of gene diagnosis in prenatal disease surveillance and screening, which will have wide application prospects.

The applications of plasma DNA in other diseases

Healthy human plasma contains only very small amounts of free DNA but the plasma DNA amounts in patients with tumor and inflammation increase significantly. In order to use plasma DNA level changes to diagnose the malignant tumor, all kinds of acute or chronic inflammation and autoimmune diseases must not be present. Studies have found that (26) the plasma DNA levels in patients who suffered burns within 24 to 48 hours were significantly higher than in the control group. This can also be used to assess the severity by the percentage of total burn surface

area, as plasma DNA levels correlated to the degree of the burns.

According to Fatouros IG *et al*. (27), the levels of plasma DNA in patients with trauma and stroke are related to the severity of the disease, whereas in athletes the levels of circulating DNA in plasma also increase with amount of exercise, especially when correlated with heavy and weight bearing exercises. The increase in levels of plasma DNA concentration may persist for several days, especially in the patients with multiple organs dysfunctional syndrome (28).

Mosca *et al*. (29) studied for correlation between the plasma DNA concentrations in patients with multiple sclerosis and disease clinical changes but found no correlation with the organ damaging location. However plasma DNA levels increased significantly during the disease activity. It is suggested that plasma DNA is also an independent risk factor for ICU mortality. The mainly reasons are blood perfusion and anaerobic glycolysis in sepsis and septic shock patients which leaded to hypoxia, which explains the phenomenon that the septicemia caused tissue damage and apoptosis in cells (30). For mild pancreatitis patients with pancreatic necrosis in the light degree, the concentration of plasma DNA was lower significantly than that in severe acute group, which shows no statistical significance when compared with the healthy control group. Therefore it can be considered that levels of the plasma DNA in patients with acute pancreatitis are associated with the accounts and severity of the disease. It can be used as a good index for clinical evaluation for patients with acute pancreatitis.

The characteristics and measurement methods of plasma DNA

In recent years, the plasma DNA and its potential application value have attracted much interest in the medical field. Applications using plasma DNA can be divided into quantitative method to measure the total plasma level and qualitative method to detect the specific gene alterations.

The method to measure the blood circulation of the content of DNA is a key step in quantitative analysis of circulating DNA

With the development of molecular biological technology, the emergence of a series of effective applications of research methods, including spectrophotometer, enzyme-linked immunosorbent assay (ELISA), quantitative real-time PCR (RQ-PCR) and single nucleotide polymorphism analysis, and greatly improve the sensitivity of detection of plasma DNA. Both RQ-PCR and ELISA methods have the capacity of determining levels of circulating DNA with a high degree of consistency and accuracy.

Although RQ-PCR has a much higher sensitivity for detecting the cfDNA, a larger volume of plasma (200 μL) is required, compared to the 15 μL required for ELISA (31). The use of SYBR green I dye (32), which binds specifically to double-stranded DNA and produces strong fluorescence, allows specific detection of circulating double stranded DNA, and is further advantageous with regards to sensitivity, time of testing and cost. It is the better method of double stranded DNA detection in blood circulation and real-time quantitative PCR is widely used for the detection of β-actin, β-globin genes in cfDNA.

The qualitative analysis about circulating DNA

Studies showed in the molecular biology, including in the mutation of ontogeny activation, inactivation of tumor suppressor genes such as *Ras* gene and *P53* gene, tumor suppressor genes methylamine, microsatellite instability (MSI) phenomena such as the LOH and DNA immunoglobulin heavy chain.

The analysis of the mutated plasma DNA in cancer genes

The methods of the detection of point mutations in plasma DNA can range from restriction fragment length polymorphism (RFLP), single strand conformation polymorphism, oligonucleotide probe hybridization and direct sequencing. The first study of plasma tumor DNA is the detection of *RAS* gene point mutation. More than 90% of pancreatic cancer and about 80% of colorectal cancer are due to mutations in the same mutation hot spot, the twelfth codon of the *KRAS* gene. By testing plasma of patients with pancreatic cancer using RFLP, the specificity of the assay was 100%, but the sensitivity was 27%, and the tumor, which especially has close relationship with the distant or not distant metastasis.

Studies have suggested (33) that tumor-associated mutations exist in healthy individual plasma arising from exposures to the carcinogens, and the presence of these mutations in cfDNA increased the risk of forming cancer. The primary technique for tumor associated mutation detection is allele specific PCR, which can detect such mutations even in the low concentrations amongst wild

type copies. The sequence data of allele specific primers can be referred to the primary tumor tissue. However, this method will miss some common mutations of tumor associated mutations. The latest technological advance uses mass spectrometry, and single allele amplification reaction to effectively identify fetal mutation in maternal plasma, and should also be suitable for mutation-specific testing in patients with tumors.

Microsatellite studies on plasma DNA

Microsatellites, also called short tandem repeats, is composed of 2-6 base pairs as the core unit, repeated 10-60 times. Its total length is usually less than 150 bp, and is widely distributed and show high levels of polymorphism across human populations. The changes of the DNA microsatellite mainly consists of MSI and allelic LOH, the latter being more common. The phenomena arise from the DNA mismatch-repair system, and microsatellite alterations in circulating DNA were not found in normal individuals.

MSI is a DNA replication error which results from defects of mismatch-repair system, allowing the number of repeats of the simple core sequence to increase or decrease. This in turn may allow the unstable microsatellite sequence, which normally do not play a regulatory role, to alter gene expression and to result in abnormal cellular proliferation and differentiation. In 1993, it was first shown that MSI is an important phenomenon in hereditary nonpolyposis colorectal cancer (HNPCC), and in sporadic colorectal cancer, MSI is regarded as a sensitive tumor detection indicator, which have been gradually accepted.

LOH mutation manifests usually an allele presenting and another allele is missing, and in heterozygous carriers of oncogenic mutations, can result in a pseudo-recessive genotype, leading to inactivation of tumor suppressor genes and cancer development and progression. The LOH in plasma DNA is absent in the healthy patient.

The first report on the use of PCR microsatellite polymorphism analysis for detecting cancer in peripheral blood of patients with DNA was made in 1996. Nakamoto *et al*. (34) studied 17 cases patients with malignant melanoma (MMM) for LOH at microsatellite loci in plasma DNA, and found that even when only four loci were tested, 70.6% of patients had at least one loci showing LOH and that LOH in plasma DNA can be used as a monitoring tool for MMM. Analysis of LOH in circulating free DNA is now commonly used, due to its high specificity and sensitivity, for screening tumor suppressor genes, and for monitoring recurrence and metastasis of tumor.

Experiments have shown that (35) with the progress of clinical tumor stage, the number of microsatellite DNA molecular marker showing LOH in plasma were all increased significantly, demonstrating the accumulation of further genetic variation with the occurrence and development of tumor.

Methylation changes of plasma DNA and disease status

In recently years, DNA methylation changes have been shown to play an important role in cancer development, either in the higher mutagenic rate at methylated bases or in the activation or inactivation of; tumor suppressor genes through methylation. In 1999, Esteller *et al*. (36) proposed that the plasma DNA in patients with cancer is hypermethylated at tumor suppress genes, as confirmed in patients with non small cell lung cancer. Subsequently, a number of experimental studies showed that practical circulation DNA plays an important role in cancer diagnosis and monitoring. At present, methylation status of tumor suppressor genes in plasma is used for cancer diagnosis, with the detection more reliable in the late stage of growth, but this method also can be used for early stage of tumor detection (37). An important mechanism of inactivation of gene expression is methylation of CpG island in the promoter region of the gene, which can also be specifically examined with the use of methylation specific PCR.

Chan *et al*. (38) used methylation-specific PCR to measure level of hypermethylation of RASSF1A in plasma DNA from patients (HCC) with hepatocellular carcinoma and chronic hepatitis B virus (HBV) carriers and found that 93% of HCC patients had hypermethylation, compared to 58% of HBV patients and only 8% in normal controls. Furthermore, If RASSF1A level is still high in patients of initial diagnosis or one year after tumor resection, survival period of patient is very poor.

Conclusions

Plasma DNA is a new type of molecular marker in disease testing with many quantitative and qualitative advantages over previous methods of testing. In recent years, the molecular detection of plasma DNA has been undertaken in the clinical setting with significant and fast progress. Not surprisingly there are some challenges we are facing from issues to be solved, including: the sample collection protocol standardisation, the sensitivity and specificity for

some special conditions, affecting factors from the abnormal liver function, effect of the latent virus infectious on plasma DNA, effect of immunological status on plasma DNA and other affecting factor.

With the rapid development of molecular biology techniques, experimental and clinical studies of plasma DNA will lead to improvements in testing in the near future and allow greater adoption of plasma DNA applications in cancer diagnosis and management, as well as numerous other diseases.

Acknowledgements

We would like to thank Dr. Gladys HO, Genetic Metabolic Disorders Research Unit, Western Sydney Genetics Program, Children's Hospital at Westmead, Sydney, NSW, Australia for her kindly help with her excellent English and editorial skills.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646-50.
2. Hohaus S, Giachelia M, Massini G, et al. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. Ann Oncol 2009;20:1408-13.
3. Hu Y, Ni H. Advance in human free circulating DNA. Yi Chuan 2008;30:815-20.
4. Ellinger J, Bastian PJ, Haan KI, et al. Noncancerous PTGS2 DNA fragments of apoptotic origin in sera of prostate cancer patients qualify as diagnostic and prognostic indicators. Int J Cancer 2008;122:138-43.
5. Silva JM, Dominguez G, Garcia JM, et al. Presence of tumor DNA in plasma of breast cancer patients: clinicopathological correlations. Cancer Res 1999;59:3251-6.
6. Schwarzenbach H, Pantel K, Kemper B, et al. Comparative evaluation of cell-free tumor DNA in blood and disseminated tumor cells in bone marrow of patients with primary breast cancer. Breast Cancer Res 2009;11:R71.
7. Kolesnikova EV, Tamkovich SN, Bryzgunova OE, et al. Circulating DNA in the blood of gastric cancer patients. Ann N Y Acad Sci 2008;1137:226-31.
8. Al Nakib M, Desbrière R, Bonello N, et al. Total and fetal cell-free DNA analysis in maternal blood as markers of placental insufficiency in intrauterine growth restriction. Fetal Diagn Ther 2009;26:24-8.
9. Zhong XY, Hahn S, Kiefer V, et al. Is the quantity of circulatory cell-free DNA in human plasma and serum samples associated with gender, age and frequency of blood donations? Ann Hematol 2007;86:139-43.
10. Rogers A, Joe Y, Manshouri T, et al. Relative increase in leukemia-specific DNA in peripheral blood plasma from patients with acute myeloid leukemia and myelodysplasia. Blood 2004;103:2799-801.
11. Jiang Y, Pan SY, Xia WY, et al. Dynamic monitoring of plasma circulating DNA in patients with acute myeloid leukemia and its clinical significance. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2012;20:53-6.
12. Hosny G, Farahat N, Hainaut P. TP53 mutations in circulating free DNA from Egyptian patients with non-Hodgkin's lymphoma. Cancer Lett 2009;275:234-9.
13. Wagner-Johnston ND, Gellert L, Gocke CD, et al. Clonal immunoglobulin DNA in the plasma of patients with AIDS lymphoma. Blood 2011;117:4860-2.
14. Zhong L, Huang WF. Better detection of Ig heavy chain and TCRγ gene rearrangement in plasma cell-free DNA from patients with non-Hodgkin Lymphoma. Neoplasma 2010;57:507-11.
15. Zhong L, Jia YQ, Meng WT, et al. The clinical significance of Ig heavy chain and TCR gamma gene rearrangement detected in free DNA in plasma in patients with non-Hodgkin lymphoma. Zhonghua Xue Ye Xue Za Zhi 2008;29:258-62.
16. He J, Wu J, Jiao Y, et al. IgH gene rearrangements as plasma biomarkers in Non-Hodgkin's lymphoma patients. Oncotarget 2011;2:178-85.
17. Chan KC, Zhang J, Chan AT, et al. Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. Cancer Res 2003;63:2028-32.
18. Musacchio JG, Carvalho Mda G, Morais JC, et al. Detection of free circulating Epstein-Barr virus DNA in plasma of patients with Hodgkin's disease. Sao Paulo Med J 2006;124:154-7.
19. Gandhi MK, Lambley E, Burrows J, et al. Plasma Epstein-Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin's lymphoma. Clin Cancer Res 2006;12:460-4.
20. Lei KI, Chan LY, Chan WY, et al. Diagnostic and prognostic implications of circulating cell-free Epstein-

Barr virus DNA in natural killer/T-cell lymphoma. Clin Cancer Res 2002;8:29-34.
21. Mussolin L, Burnelli R, Pillon M, et al. Plasma cell-free DNA in paediatric lymphomas. J Cancer 2013;4:323-9.
22. Wagner-Johnston ND, Gellert L, Gocke CD, et al. Clonal immunoglobulin DNA in the plasma of patients with AIDS lymphoma. Blood 2011;117:4860-2.
23. Greystoke A, O'Connor JP, Linton K, et al. Assessment of circulating biomarkers for potential pharmacodynamic utility in patients with lymphoma. Br J Cancer 2011;104:719-25.
24. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
25. Fu XH, Chen HP. Advances on circulating fetal DNA in maternal plasma. Chin Med J (Engl) 2007;120:1256-9.
26. Fox A, Gal S, Fisher N, et al. Quantification of circulating cell-free plasma DNA and endothelial gene RNA in patients with burns and relation to acute thermal injury. Burns 2008;34:809-16.
27. Fatouros IG, Destouni A, Margonis K, et al. Cell-free plasma DNA as a novel marker of aseptic inflammation severity related to exercise overtraining. Clin Chem 2006;52:1820-4.
28. Lam NY, Rainer TH, Chan LY, et al. Time course of early and late changes in plasma DNA in trauma patients. Clin Chem 2003;49:1286-91.
29. Mosca M, Giuliano T, Cuomo G, et al. Cell-free DNA in the plasma of patients with systemic sclerosis. Clin Rheumatol 2009;28:1437-40.
30. Saukkonen K, Lakkisto P, Pettilä V, et al. Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. Clin Chem 2008;54:1000-7.
31. Holdenrieder S, Stieber P, Chan LY, et al. Cell-free DNA in serum and plasma: comparison of ELISA and quantitative PCR. Clin Chem 2005;51:1544-6.
32. Anker P, Mulcahy H, Stroun M. Circulating nucleic acids in plasma and serum as a noninvasive investigation for cancer: time for large-scale clinical studies? Int J Cancer 2003;103:149-52.
33. Hagiwara N, Mechanic LE, Trivers GE, et al. Quantitative detection of p53 mutations in plasma DNA from tobacco smokers. Cancer Res 2006;66:8309-17.
34. Nakamoto D, Yamamoto N, Takagi R, et al. Detection of microsatellite alterations in plasma DNA of malignant mucosal melanoma using whole genome amplification. Bull Tokyo Dent Coll 2008;49:77-87.
35. Eisenberger CF, Stoecklein NH, Jazra S, et al. The detection of oesophageal adenocarcinoma by serum microsatellite analysis. Eur J Surg Oncol 2006;32:954-60.
36. Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999;59:67-70.
37. Hoque MO, Feng Q, Toure P, et al. Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer. J Clin Oncol 2006;24:4262-9.
38. Chan KC, Lai PB, Mok TS, et al. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. Clin Chem 2008;54:1528-36.

Cite this article as: Wang S, Chen Y, Wu Z. Advances in the medical research and clinical applications on the plasma DNA. Transl Pediatr 2014;3(2):140-148. doi: 10.3978/j.issn.2224-4336.2014.03.07

