CIRCULATING DNA

PROMISING NON INVASIVE MARKERS FOR CANCER RESEARCH

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Abstract

DNA is the repository of genetic information in each living cell, its integrity and stability is essential to life. Small amounts of free DNA circulate in healthy individual, variable amount in many pathological condition with increased concentrations of DNA are present in the plasma of cancer patients. Tumor DNA in blood represents a promising value in clinical medicine for early diagnosis, prognosis and monitoring of therapy. Several studies have identified DNA genetic alterations in circulating plasma DNA from cancer patients that match with genetic changes present in primary tumors and it has been postulated that tumor necrosis causes release of DNA of varying sizes, which contrasts apoptosis in normal tissue that releases smaller and more uniform DNA fragments are of great value for clinical diagnosis. Circulating DNA may be clinically useful used as surrogate for many clinical application and research.

Key word
DNA, DNA concentration, DNA integrity, DNA genetic alteration, Cancer

Abbreviation
cc-DNA; Circulating cancer DNA, cf-DNA; Cell free DNA, cf-NA; circulating free nucleic acid, ct-DNA; Circulating tumor DNA

Introduction

Nucleic acid in blood, particularly DNA has been suggested as a promising value for clinical application in clinical medicine for early diagnosis, prognosis and monitoring of therapy has been a significant advancement in the field. Whether the DNA is present in normal locations such as the nucleus and mitochondria or circulating free in the blood and body fluids, it can be utilized as a valuable biomarker. Circulating DNA or cell free DNA (cf-DNA) from blood is easily accessible, reliable, and reproducible.

A number of sources appear to contribute to circulating nucleic acid; the presence of DNA and RNA circulating freely in the blood stream of healthy subjects: apoptotic cell death is the major source which is the normal process in physiological conditions as well as in a non-cancer pathological condition, e.g. cirrhosis or actively release nucleic acids in the blood circulation.

In healthy individuals, small amount of circulating DNA, since most non-living cells are removed efficiently from circulation by phagocytes. The circulating DNA in plasma is protein-bound (nucleosomal) DNA and circulating DNA has a short half-life (10 to 15 min) to several hours which is removed mainly by the liver.

Molecular weight cf-DNA may indicate its source; apoptotic cells undergo programed enzymatic cleavage and release uniformly DNA fragments mostly 185 to 200 (up to1000) bp in size, the fragments tend to form a ladder pattern When

double stranded circulating DNA in plasma and serum is separated by gel electrophoresis, whereas in necrosis; DNA incompletely and non-specifically digested results in higher molecular weight fragments and thus smears on electrophoretic separation due to its fragment sizes up to 10,000.

A correlation between elevated levels of cf-DNA and several disorders has been established since the 1950s, high amount of cf-DNA can be found in the circulation of patients with rheumatoid disease, myocardial infarction, inflammatory diseases, sepsis, blunt trauma, burns, autoimmune disease, or stroke and also with cancers. In patients affected by neoplastic diseases, circulating free nucleic acid in blood (cf-NA) increased because cancer cells that detach from the tumor mass and undergo necrosis due to necrotic processes characteristic of tumor cell.

Accumulation of DNA in the circulation can result from an excessive release of DNA caused by massive cell death, inefficient removal of the dead cells, or a combination of both. The reduced clearance of cf-DNA caused by impaired organ function during systemic inflammation may also contribute to cf-DNA elevation in the blood of patients with many diseases. The persistence of DNA in the circulation could be due to the function of DNAse I and II in the serum. In malignant disease, lower activity of these enzymes has been reported. This may be due to the release of an inhibitor which may be present in tumors cells.

Circulating DNA

DNA is a nuclear macromolecule that can exist in an intracellular and extracellular form. The presence of circulating cell-free DNA has been discovered as early as the 1940's, its extracellular form, can appear circulating in the blood as well as present in other biological fluids. Small amounts of free DNA circulate in healthy individual, variable amount in many pathological condition could be detected in patients with benign lesions, inflammatory diseases, cirrhosis with increased concentrations of DNA are present in the plasma of cancer patients.

Circulating Cancer DNA (cc-DNA) or **Circulating Tumor DNA (ct-DNA)** is a part from cf-DNA; generally, cc-DNA constitutes only a minor fraction (less than 1%) of the cf-DNA although the actual fractions can range depending on the tumor size, location and vascularity, and the disease extent and stage, the cells release DNA due to alteration of their membrane permeability whether they are at their primary location or circulating in the peripheral blood.

cc-DNA is formed by non-uniformly DNA fragments, due to necrotic processes characteristic of tumor cell, these fragments are gathered from all tumor sites into circulation; thus, they reflect heterogeneity of the entire tumor.

Applications of Circulating DNA For Cancer Research

For cancer patients, a variable amount of circulating DNA will come from the tumor (ct-DNA), opening the possibility to perform sequential genomic characterization of patient's cancer.

The use of circulating DNA would be to diagnosis and monitor for disease recurrence of an individual and as a prognostic tool to detect early stage cancers that may be amenable to treatment or surgical resection and cure.

Detection of Tumor Specific Genetic Markers

The detection of tumor specific genetic or epigenetic markers characteristics of tumor DNA have been found in genetic material extracted from the plasma of cancer patients; specific genetic alterations of certain malignancies are useful in the detection or identifying, including, increased expression of specific oncogene or tumor suppressor gene, mutational events, microsatellite alterations, gene's methylation, and other genetic aberrations.

Point mutations of the ras genes have been detected in the plasma DNA of patients suffering from haematopoetic malignancies, colorectal and pancreatic cancer, sometimes prior to clinical diagnosis.

- Rearranged immunoglobulin heavy chain DNA has been found in the plasma of patients with non-Hodgkins lymphoma and acute B cell leukaemia.
- Microsatellite instability in the plasma and serum DNA of patients suffering from head and neck, lung and renal cell cancer.
- Analyses of cf-DNA for mutations conferring response or resistance to targeted therapies have been demonstrated in several studies on advanced-stage cancer e.g. mutations of the EGFR gene in lung cancer, mutations of the K-RAS gene in colorectal cancer, TP53 and PIK3CA mutations in breast cancer, but recently, more tests for mutations in genes encoding therapeutic targets or the corresponding resistance genes will follow in the near future, especially, NSCLC.

With recent technological advancements, highly sensitive techniques are available for the detection and analysis of mutant alleles present in the circulation at very low frequencies.

Circulating DNA Concentration and Integrity

DNA Concentration

Several studies have focused on measuring the absolute circulating DNA concentration. Circulating cell free DNA concentration draw an attention; although, it is difficult to draw any firm conclusions about blood levels of DNA from many studies since a variety of different methodologies were used for DNA purification by different laboratories.

In healthy subjects, plasma DNA concentration levels is extremely variable ranging from 0 to 100 ng/ml of blood with the average concentration of cf-DNA was of 30 ng/ml, while in cancer patients, the DNA concentration was reported to range from zero to microgram levels with a mean of 180± 38 ng/ml; 0- 50 ng/ml in 50% of patients, 50- 5000 ng/ml in another 50% of patients. Higher levels in cancer patients with the highest levels were observed in patients with advanced metastatic disease, persistently elevated levels of cf-DNA were associated with resistance to treatment.

In cancer patients, many studies have focused on total DNA concentration and most circulating DNA studies focused on advanced-stage cancers with relatively high concentrations of cf-DNA, detailed experiences with early-stage cancer with low concentrations of cf-DNA are lacking; In pathological conditions, elevated levels of cf-DNA are present in various pathological conditions, high levels of cf-DNA, also aggravated during inflammation and injury and other non- progressing benign lesions; the broad prevalence of diseases with potentially elevated cf-DNA levels limits, to a certain extent, the diagnostic specificity, further studies are needed.

Limitation to the use of plasma DNA concentration; no cutoff value for plasma DNA concentration produced performance characteristics that make it a good screening tool for neoplastic disease and the circulating DNA values significantly vary in different cancers and other diseases, which restrict plasma DNA

concentration tests as a general tool for cancer diagnosis and plasma DNA levels were not elevated in some neoplastic diseases, even though large tumors may be associated with low plasma DNA concentration; this might be attributed to minimal cell death or to high clearance rate caused by short half-life of plasma DNA or DNAase enzyme activity.

Furthermore, cf-DNA concentration is not specific for cancer, it is increased in various benign neoplasm and various diseases, The circulating DNA values significantly differ in different cancer which restricts concentration of plasma DNA as a general tool for cancer diagnosis, but it may have different diagnostic or prognostic value in different cancers. The concentration of cf-DNA alone seems not to be sufficient for high diagnostic performance or to be used as a predictive biomarker further refinement is needed.

DNA Integrity as a Cancer Biomarker

DNA fragmentation could be used as a cancer biomarkers. Recently, the ratio of longer DNA and shorter DNA called DNA integrity index was found to be increased in cancer patients; the aim of identifying its origin (tumor or healthy cells).The DNA integrity index was originally characterized in DNA isolated from stool samples as an indication of the presence of colorectal carcinoma, the integrity of cf-DNAs has been shown to be altered in different cancers including colorectal carcinoma (CRCs), testicular, nasopharyngeal, and ovarian cancer.

Many works stated the possibility to discriminate ct-DNA from circulating DNA by analyzing its size distribution.

DNA released from malignant cells varies in size because pathologic cell death in the malignant tumors results not only from apoptosis, but also necrosis, autophagy, or mitotic catastrophe. Tumor necrosis is a frequent event in solid malignant neoplasms, and it generates a spectrum of DNA fragments with different strand lengths because of random and incomplete digestion of genomic DNA by a variety of deoxyribonucleases. Therefore, elevated levels of long DNA fragments may be a good marker for detection of malignant tumor DNA in blood

DNA Assay Standardization

Circulating DNA may be a promising value for diagnosis and prognosis but assessment of the free circulating DNA is not yet practical for clinical use, the DNA assay needs scrupulous standardization, a potential pre-analytic limitation may be the purification of DNA from serum or plasma especially with tumor DNA, which decreases DNA yield, other important variables have included time between collection and processing of samples in addition to storage prior to performing the assay, freeze-thawing of samples and the effects on DNA stability. The importance of reliable collection, high quality DNA, and storage procedures will ensure comparable quality of samples and thus efficient genomic analysis of such samples within laboratories, because, the discrepancies in sensitivities across various studies may be attributed to technical issues, the

sequences and choice of primers, the amplification process, and the DNA quantitation methods, the sensitivity and specificity of these methods have not been validated as the standardization of sample processing and also preferred source of cf-DNA has not been established yet, the source material of cf-DNA should be considered.

Conclusion

Specific genetic change; these approaches challenging for clinical applications has the highest importance for cancer diagnosis or individualization for therapy for each cancer types or for detection of broad spectrum specific genetic markers for various cancer type; Thus, presence of tumor-specific alterations remain the best criterion to assess the tumor origin of cc-DNA, evaluating cc-DNA been proposed that the kinetic monitoring of circulating genetic markers could allow to trace the evolution of the tumor, to determine the effectiveness of a treatment or to detect any potential recurrence, further validation studies crucially needed

The circulating DNA values significantly differ in different cancer which restricts concentration of plasma DNA as a general tool for cancer diagnosis, Also other diseases such as systemic lupus erythematosus, rheumatoid arthritis, pulmonary embolism and myocardial infarction have been linked to elevated DNA levels but it may have different diagnostic or prognostic value in different cancers. No cutoff value for plasma DNA concentration established to produce performance characteristics that make it a good screening tool for neoplastic disease but,

elevated levels of long DNA fragments may be a good marker for detection of malignant tumor DNA in blood, further studies crucially needed.

Development of new genomics technologies such next generation sequencing (NGS) or array comparative genomic hybridization (aCGH) has greatly increased our knowledge of tumor development at the molecular level. Moreover, the characterization of patient samples for the discovery or tracking of genetic biomarkers has been greatly expanded.

Conflicts of interest

The author declares that there are no conflicts of interest.

References

[1]- Wang BG, Huang HY, Chen YC, Bristow R E, et al. Increased plasma DNA integrity in cancer patients. Cancer research 2003; 63(14): 3966-8.

[2]- Gormally E , Caboux E , Vineis P , Hainaut P. (2007). Circulating free DNA in plasma or serum as biomarker of carcinogenesis. Practical aspects and biological significance. Mutation Research. Mutation research; 635:105-11

[3]-Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989; 46: 318–22.

[4]- Garcia DC, Ruiz P R, GarciaOD. Circulating nucleic acids in plasma and serum (CNAPS) and its relation to stem cells and cancer metastasis: state of the issue. Histol Histopathol 2004; 19:575–83.

[5]- Taback B, Hoon DS. Circulating nucleic acids and proteomics of plasma/serum: clinical utility. Ann N Y Acad Sci 2004;1022: 1–8.

[6]- Kawakami K, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, et al. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. J Natl Cancer Inst 2000; 92 :1805–11.

[7]- Fleischhacker M, Schmidt B. Free circulating nucleic acids in plasma and serum useful for the detection of lung cancer patients? Cancer Biomark. 2010; 6(3-4):211-9.

[8]-Watson J.D. and Crick F.H.C. A Structure for Deoxyribose Nucleic Acid**.** *Nature* 1953; **171**, 737-738.

[9]- MirskyAE,1968. The discovery of DNA. Scientific American 218 (June):77-88.

[10]- Koffler D, Agnello V, Winchester R, Kunkel HG. The occurrence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. J Clin Invest 1973; 52: 198-204.

[11]- Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983; 51: 2116-2120.

[12]-Giacona MB, Ruben GC, Iczkowski KA, Roos TB, Porter DM, Sorenson GD. Cell-free DNA in human blood plasma: Length measurements in patients with pancreatic cancer and healthy controls. Pancreas 1998; 17: 89-97.

[13] -Wu TL, Zhang D, Chia JH, Tsao KH, Sun CF, Wu JT. Cell-free DNA: measurement in various carcinomas and establishment of normal reference range. Clin Chim Acta 2002; 321: 77-87.

[14] -Sozzi G, Conte D, Leon M, Ciricione R, Roz L, Ratcliffe C, *et al*. Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol 2003; 21: 3902-3908.

[15]- Allen D, Butt A, Cahill D, Wheeler M, Popert R, Swaminathan R. Role of cell-free plasma DNA as a diagnostic marker for prostate cancer. Ann N Y Acad Sci 2004; 1022: 76-80.

[16]- Jung K, Stephan C, Lewandowski M, Klotzek S, Jung M, Kristiansen G, *et al*. Increased cell-free DNA in plasma of patients with metastatic spread in prostate cancer. Cancer Lett 2004; 205: 173-180.

[17]- Papadopoulou E, Davilas E, Sotiriou V, Koliopanos A, Aggelakis F, Dardoufas K, *et al*. Cell-free DNA and RNA in plasma as a new molecular marker for prostate cancer. Oncol Res 2004; 14: 439-445.

[18]- Taback B, O'Day SJ, Hoon DS. Quantification of circulating DNA in the plasma and serum of cancer patients. Ann N Y Acad Sci 2004; 1022: 17-24