DNA INTEGRITY USES AND CLINICAL UTILITY IN CLINICAL MEDICINE

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Introduction

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 both healthy and diseased human plasma/serum and increased concentrations of DNA are present in the plasma of cancer patients. Tumor released DNA in blood represents a promising biomarker for cancer detection. Several studies have identified DNA 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. DNA integrity index represented by the ratio of longer fragments to shorter DNA; may be clinically useful used as surrogate for detection of tumors.

Introduction

DNA is a nuclear macromolecule that can exist in an intracellular and extracellular form. In its extracellular form, DNA can appear in the blood as well as other biological fluids. Small amounts of free DNA circulate in both healthy and diseased human plasma/serum, and increased concentrations of DNA are present in the plasma of cancer patients and the analyses of plasma DNA alterations may theoretically be used for prognostic purposes or for early diagnosis and other detection strategies. Characteristics of tumor DNA have been found in genetic material extracted from the plasma of cancer patients; these features include decreased strand stability and the presence of specific oncogene, tumor suppressor gene and microsatellite alterations. 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, expressed either as a new allele or a loss of one allele (LOH) occurs in the plasma and serum DNA of patients suffering from head and neck, lung and renal cell cancer.

Jahr et al. proposed that DNA could be released from apoptotic or necrotic cells and that DNA size distribution may be used to determine the origin of DNA from either apoptotic or necrotic cells.

Cell Free DNA (Circulating DNA)

The presence of circulating cell-free DNA has been documented as early as the 1940's (Mandel and Metais, 1947)**.** A baseline level of circulating DNA is generally present, even in healthy individuals. Long DNA molecules broken into shorter fragments and a portion of these fragments find their way into the blood stream, A number of sources appear to contribute to circulating cell-free DNA; it arise from dying cells break up within every organ of the body, cells of a developing fetus, or a tumor cells that may be growing somewhere, it contains genetic information from all cell types of the body. Among the many DNA fragments are those that carry recognizable signatures that identify their origin such as an undiagnosed tumor; however it was not until the 1970's that a correlation between elevated levels of circulating DNA and several human disorders, including cancer . The elevated levels of circulating DNA observed in cancer patients by researchers may be related to neoplastic cells, however this was not verified until the 1990's, when several reports were published showing that circulating DNA exhibited various tumor-like alterations. While levels of circulating DNA may be related to natural cell turnover and cell lysis of healthy cells and neoplastic cells, it is unlikely all circulating DNA can be attributed to cell lysis/apoptosis. Approximately ten thousand tumor cells would need to be circulating per millilitre of blood in order to produce the reported elevated cell-free DNA levels, and this number of cells has never been seen. Circulating DNA can

be identified consistently in patients with neoplastic diseases compared with healthy controls.

The use of circulating DNA would be to monitor for disease recurrence or to establish disease free status of an individual and as a prognostic tool to detect early stage cancers that may be amenable to treatment or surgical resection and cure. Variations in the sample type and processing techniques were shown to affect the quantity of cell free DNA rescued. While no correlation has yet been found between circulating DNA levels and the size or location of the primary tumor, circulating DNA has already been proposed by scientists for diagnostic and prognostic applications.

Many results suggested that plasma DNA concentration may not be sensitive or specific enough for cancer screening or diagnosis because it is difficult to eliminate the influence of circulating DNA concentration variance among different kinds of cancers which restrict plasma DNA as a general tool for cancer diagnosis, but it may have different diagnostic or prognostic value in different cancers. Also other conditions such as pregnancy, systemic lupus erythematosus, rheumatoid arthritis, pulmonary embolism and myocardial infarction have been linked to elevated DNA levels.

Although circulating DNA from cancer patients were found to have decreased strand stability, oncogene and tumor suppressor alterations, microsatellite alterations, p53 mutations, and many other characteristics in common with neoplastic cells**.** The low sensitivities reported for detection of DNA alterations in plasma make these approaches challenging for immediate clinical applications.

Brief summary of the cancer types that are positively correlated with elevated levels of circulating DNA (http://www.thecancertest.com/science-of-cell-free-dna/)

DNA Integrity Index

DNA integrity index as represented by the ratio of longer fragments to shorter DNA may be clinically useful used as surrogate for detection of tumors. In

healthy individuals, the main source of free-circulating DNA is apoptotic cells. Apoptotic cells release DNA fragments that are usually 185 to 200 base pair (bp) in length; this uniformly truncated DNA is produced by a programmed enzymatic cleavage process during apoptosis. In contrast, 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, however, quantification of free circulating DNA in blood has not been practically utilized because of difficulty in handling the minute amount of DNA.

The DNA integrity assay (DIA) was originally characterized in DNA isolated from stool samples and was shown to be indicative of colorectal tumor presence. Subsequent experiments have shown that large fragments of DNA were released from tumor cells via tumor necrosis facilitating the maintenance of DNA integrity. In contrast, cell death in normal tissues is closely regulated by apoptosis in which the processing of DNA results in fragments between 180 and 210 bp. Hence, DIA is a qualitative rather than a quantitative assay. The potential differential processing of DNA in normal and cancer states has been explored in the plasma of females with cancer, presenting the first indication that DIA may provide a simple measure for the detection of cancer. The size of circulating DNA has been shown to be increased in patients suffering from gynecologic, colorectal, breast, and head and neck cancers when compared with healthy subjects.

Brief summary of the cancer types that are positively correlated with increased DNA integrity index.

Authors (year)	Cancer Type	Results
Boynton	Colon	DNA fragments isolated from CRC patients were of higher

2003	Cancer	molecular weight (>18 bands detected of a total of 24 possible bands) than fragments isolated from fecal DNA of the colonoscopy-negative control group.
Wang *et al.*(2003)	Gyn.& breast	The findings suggest that increased DNA integrity in plasma DNA is associated with cancer
Jiang et al. (2006)	Head &Neck	This study shows that DNA integrity index in the plasma of the patients with HNSCC is increased in comparison with that in the plasma from non-HNSCC control subjects
Robert Hanley	Prostate Cancer	Patients with prostate cancer (86 of 123; 69.9%) were determined to have a positive DIA score of ≥7.
Umetani*et al.*(2006)	Prostate Cancer	Patients with prostate cancer (86 of 123; 69.9%) were determined to have a positive .

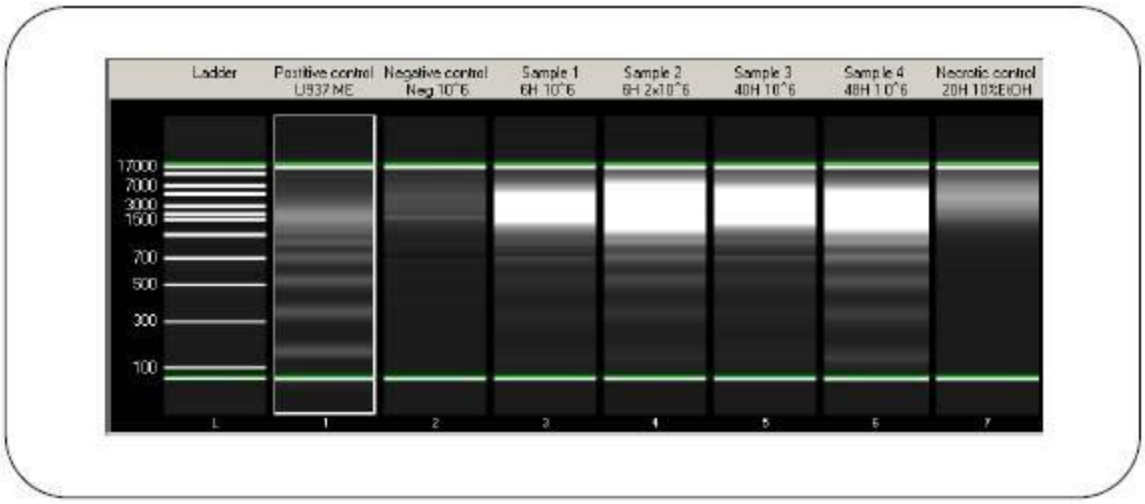

Figure 4
Gel-like image showing positive and negative controls together with 4 apoptotic samples. Negative and necrotic samples display an overall smaller DNA signal and no trace of laddering.

DNA integrity index may be a promising tumor marker for diagnosis and prognosis but assessment of the integrity of free circulating DNA is not yet practical for clinical use because the sensitivity and specificity of these methods have not been validated. A potential limitation may be the purification of DNA from serum or plasma, which decreases DNA yield. DNA loss may be inversely dependent on fragment size, which would affect DNA integrity values. Other important variables have included time between collection and processing of samples in addition to storage prior to performing the assay, i.e., freeze-thawing of samples and the effects on DNA stability. Although many research reported many cancer types that are positively correlated with increased DNA integrity index. In contrast discrepancies of various result reported; Holdenrieder et al. 2008 found that DNA concentrations were not different in plasma (median: 4.5 ng/mL) and serum (67.1 ng/mL) of patients with benign diseases when compared with values in plasma (5.1 ng/mL) and serum (65.4 ng/mL) of cancer patients. Similarly, the DNA integrity index in plasma (0.38), and serum of patients with benign diseases (0.29) was comparable to values in plasma (0.33) and serum (0.37) of cancer patients. In conclusion, they could not confirm a high diagnostic utility of the DNA integrity index. However, a combination with other markers such as DNA may enhance sensitivity for cancer detection. Schmidt et

al., 2008 conclude that DNA integrity index method is not useful in a diagnostic setting and is not able to differentiate between lung cancer patients and patients with a benign lung disease.

DNA integrity index analysis

DNA strand integrity was measured by quantitative PCR (QPCR) using the real-time system most commonly. Plasma/serum DNA was tested by amplifying house keeping gene e.g. β-actin, Both longer and shorter bp products were amplified and the integrity index calculated as the ratio between them.

Recently, a robust, highly sensitive, high-throughput method was developed to measure the integrity of free circulating DNA in serum by qPCR for ALU repeats. The ALU is the most abundant repeated sequence in the human genome, with a copy number of ~1.4 x 10^6 per genome. ALU sequences are short interspersed elements, typically 300 nucleotides in length, that account for more than 10% of the human genome . ALU elements multiply within the genome in a retroposition process through RNA polymerase III–derived transcripts from evolution therefore; qPCR of ALU repeats with a properly designed primer set can dramatically increase the sensitivity of size-dependent DNA measurement.

Summary and Conclusion

- 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 low sensitivities reported for detection of DNA alterations in plasma, however, make these approaches challenging for immediate clinical applications.
- Existing Data on the relationship between DNA integrity index and cancer are limited.
- Due to lack of current research and discrepancies of various results; further validation studies are needed to assess the feasibility of using

plasma DNA integrity index as a screening tool for detection of malignancy and/or tumor progression.

- DNA integrity index, simple; non-invasive approach is important for the future of cancer. Using DNA integrity index may be a promising tumor marker for diagnosis and prognosis and follow-up tracking and could complement to established markers with especial emphasis on stringent control over the variables associated with sample collection and processing.

Conflicts of interest

The author declares that there are no conflicts of interest.

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