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Circulating nucleic acids in plasma or serum

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Abstract

Background: Nucleic acids can be found in small amounts in healthy and diseased human plasma/serum. Higher concentrations of DNA are present in the plasma of cancer patients sharing some characteristics with DNA of tumor cells. Together with decreased strand stability, the presence of specific oncogene or tumor-suppressor gene mutations, microsatellite alterations, Ig rearrangements and hypermethylation of several genes may be detected. Moreover, tumor-related mRNA has been found circulating in the plasma/serum. *Conclusions*: The results obtained in many different cancers have opened a new research area indicating that circulating nucleic acids might eventually be used for the development of noninvasive diagnostic, prognostic and follow-up tests for cancer. © 2001 Published by Elsevier Science B.V.

Keywords: Circulating DNA; Plasma/serum; Cancer; Diagnostic

1. High amounts of DNA are found in plasma/serum of cancer patients

In 1977, Leon et al. [1] reported that cancer patients harbored in their plasma higher levels of circulating DNA compared to healthy controls. Moreover, greater amounts of DNA were found in the plasma/serum of patients with metastases compared to those with localized disease. Interestingly, DNA levels decreased by up to 90% after radiotherapy, while persistently high or increasing DNA concentrations were associated with a lack of response to treatment.

After having extracted and purified the DNA from the plasma, we found in various malignancies (leukaemia, lymphoma, lung, breast and gastrointestinal tumors) that detectable amounts of circulating DNA were found only in patients with advanced malignancies bearing a large tumor cell burden [2].

2. DNA in the plasma/serum of cancer patients share biophysical properties with DNA of cancer cells

Increased levels of plasma DNA were found in cancer patients but it was not determined if the circulating DNA was released from activated lymphocytes reacting towards the disease or from the tumor cells themselves. Our laboratory was able to show in 1989 that this plasma DNA from cancer patients shared some biophysical properties (decreased strand stability) common to DNA of cancer cells and, hence, was of tumoral origin [2]. This approach was interesting but it required microgram

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amounts of DNA. Luckily, PCR techniques became available allowing oncogene mutation detection in minute quantities.

3. Tumor-related alterations in plasma/serum of patients with hematological malignancies

N-RAS mutations have been found in DNA extracted from the bone marrow of patients with myelodysplastic syndrome and acute myelogenous leukaemia (AML). These alterations have been also found in the plasma, leukocytes and bone marrow of such patients [3]. In patients with N-RAS alterations, mutant DNA was always present in plasma DNA, though sometimes absent in the DNA of peripheral blood cells or bone marrow indicating that a single bone marrow biopsy or aspiration does not necessarily contain all the malignant clones involved in the disease.

4. Rearranged Ig in plasma DNA as a marker for B cell malignancy

Another study on hematological disorders was done on B-cell malignancies where rearranged Ig heavy chain DNA was detected in plasma or serum samples of patients [4]. Tumor-derived clonal CDRIII DNA was found in serum or plasma in 47% of 110 patients with non-Hodgkin's lymphoma or acute B-precursor lymphoblastic leukaemia. Follow-up showed a close correlation between persisting tumor-derived plasma/serum DNA and resistant disease or early relapse.

5. *K-RAS* mutations in plasma DNA of colorectal cancer patients

Several publications [5,6] have reported the presence of *K-RAS* mutations in the circulating DNA corresponding to the mutation found in the tumor. In one of these studies, the authors have been able to follow-up a number of patients after surgery [7]. Post operatively, *K-RAS* mutations were found in the serum of five patients, two of which developed a relapse. The plasma mutation detection predated the clinical recurrence by 1 year in one case.

6. *K-RAS* mutations in plasma DNA of pancreatic cancer patients

The *K-RAS* gene is mutated in approximately 90% of pancreatic adenocarcinomas, suggesting that the analysis of many genes would be unnecessary to detect the majority of cases. Several studies have, therefore, also been focused on the detection of this mutation in the plasma of pancreatic cancer patients [5,6].

In one study [8], plasma DNA was isolated from 21 pancreatic cancer patients and *K-RAS* alterations was detected in the plasma of 17 patients (81%). In cases where both plasma and pancreatic tissue were available, DNA mutations were similar in corresponding plasma and tissue samples. Plasma DNA alterations were found 5–14 months before clinical diagnosis in four patients who had been diagnosed as suffering from pancreatitis showing again that circulating tumor DNA may be an early event in oncogenesis. Mutant DNA was not found in the plasma of three patients with chronic pancreatitis who did not develop a carcinoma.

A follow-up study of patients with pancreatic adenocarcinoma also resulted in plasma DNA alterations being detected in a high proportion of cases in which a *K-RAS* mutation was found in tumor tissue [9]. Treatment resulted in disappearance of *K-RAS* gene mutations in plasma DNA in six of nine (67%) patients. Three patients with a persistently positive *K-RAS* gene mutation in pre- and post-treatment plasma samples showed early recurrence or a progressive disease.

7. Microsatellite alterations in circulating DNA of cancer patients

All tumors, however, do not have high mutation rates on easily testable hot spots. This is why several groups have looked for microsatellite alterations in the plasma/serum DNA of cancer patients. Microsatellite DNA is composed of simple repeats of unknown function. It is unstable in cancer cells and

subject to alterations, which appear as new alleles, allele expansion or loss of heterozygocity (LOH). Microsatellite DNA alterations are part of neoplastic progression and they may serve as clonal markers.

We initiated a study to detect microsatellite alterations in paired samples of plasma and tumor DNA from patients with small cell lung carcinoma compared to the same repeat sequences of normal cells from the same patients. A microsatellite alteration was present in 16/21 (76%) SCLC tumors and in 15 out of 21 (71%) corresponding plasma samples [10]. Microsatellite alterations were also found in the circulating DNA of head and neck cancer patients [11]. In this study, positive serum samples appeared to be related to patients with advanced disease, suggesting they may prove useful as prognostic factors. Microsatellite alterations have also been detected in the circulating DNA of patients suffering from a variety of malignancies: non-small cell lung [12], renal [13], bladder [14], breast [15], HNPCC and sporadic colon [16], ovarian cancers [17] and melanoma [18].

8. Free Epstein–Barr Virus (EBV) DNA in circulating DNA of patients with nasopharyngeal cancer

EBV sequences have been detected in the plasma of patients suffering from nasopharyngeal cancer [19]. Thus, viral DNA may serve as a tumor marker for a malignancy, which is widely distributed in Southern Asia. Quantitative analysis of circulating EBV can be related to prognosis.

9. Aberrant methylation of genes in plasma/serum of cancer patients

Epigenetic changes common in many kinds of malignancies may also be detected in the plasma or serum of cancer patients particularly promoter hypermethylation of several genes. Among these genes, *p16* has been specially studied. It is known that *p16* mutations are rare but promoter hypermethylation resulting in gene inactivation are found to be common in a variety of different primary tumors. New markers are available since multiple genes are now known to be somatically silenced by promoter hypermethylation. Twenty-two patients with non-small cell lung cancer were tested [20], searching for promoter hypermethylation of the tumor suppressor gene *p16*, the putative metastasis suppressor gene death-associated protein kinase, the detoxification gene glutathione *S*-transferase P1, and the DNA repair gene *O*6-methylguanine-DNA-methyltransferase. Aberrant methylation of at least one of these genes was detected in 15 of 22 (68%) NSCLC tumors but not in any paired normal lung tissue. In these primary tumors with methylation, 11 of 15 (73%) samples also had abnormal methylated DNA in the matched serum samples. Similarly, in the study of liver cancer [21], *p16* methylation was found in the plasma/serum samples of 81% (13/16) of patients presenting *p16* methylation in their tumor. Hypermethylation of *p16* was also reported in tumor and plasma DNA of a series of breast [22] and head and neck patients [23].

10. Circulating mRNA in serum of cancer patients

RNA has also been found circulating in the plasma of normal subjects and cancer patients. Tyrosinase messenger RNA (mRNA) has been extracted from the serum of melanoma patients and subjected to RT-PCR [24]. Moreover, the presence of cell-free Epstein–Barr virus-associated RNA has also been reported in the plasma of patients with nasopharyngeal carcinoma [25]. The two human telomerase RNA subunits, telomerase RNA template (hTR) and its catalytic component (hTERT) have also been detected in the serum of patients with breast or with colorectal cancer. hTR and hTERT were undetectable in tissues and sera taken from patients with benign disease and in the sera of normal subjects [26]. Thus, it is surprising that tumor-derived mRNA is not immediately broken down in the blood stream and may be found even in patients with localized disease.

11. Medical implications

From the clinical point of view, the results reported in this short review are still preliminary.

Circulating DNA opens, however, a new area of research and offers the possibility of noninvasive test for cancer diagnosis. Moreover, the association between tumor-related plasma DNA and tumor stage, sometimes observed, suggests that it might potentially serve as a prognostic marker. The first practical application probably resides in the possibility to follow-up by a noninvasive test after surgery or therapy, predicting recurrence or assessing success of treatment.

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