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Review

Circulating Cell-Free DNA in Plasma/Serum of Lung Cancer Patients as a Potential Screening and Prognostic Tool

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Background: **Evaluation of tumor-specific circulating DNA in plasma/serum is a promising noninvasive diagnostic and prognostic tool requiring only a blood sample, which circumvents the logistic difficulties associated with the use of invasive procedures for serial tumor samplings during screening of lung cancer.**

Methods: **We reviewed English-language reports published in the MEDLINE database that provided the results of qualitative and quantitative studies on circulating DNA in serum/plasma of lung cancer patients. We searched the bibliographies of the retrieved reports and reviews. Abstracts presented at the 2003, 2004, and 2005 annual meetings of the American Society of Clinical Oncology were also reviewed.**

Results: **More than 80 citations were retrieved, of which 33 met our criteria: 22 qualitative studies, and 11 quantitative studies. The studies varied in the choice of markers, frequency of alterations, sensitivities of used methodologies, sample sizes, treatment protocols, and prognostic correlates evaluated.**

Conclusions: **The findings from the studies on serum/plasma DNA suggest that it would be possible to develop a simple blood test with high sensitivity and specificity that has potential for screening of high-risk individuals, for prognostic or staging purposes, and to be used as intermediate end-points of efficacy in chemoprevention and therapeutic trials. However, further work is needed to identify additional biomarkers and to standardize present techniques for sample collection, processing, and analysis. Large prospective studies with long follow-ups are essential to eventually integrate blood marker–based assays into the clinical setting.**

Lung cancer is the leading cause of cancer death worldwide. Although the low 5-year survival rate (<15%) has changed minimally in the last 25 years, new agents are being identified to improve results. The poor outcome is attributable to the fact that almost two thirds of cases are diagnosed when locoregional and/or metastatic extension has already occurred *(1)*. A sensitive detection method could help early diagnosis and improve survival of lung cancer patients.

With the introduction of PCR-based technologies in 1980s and refinements thereof, numerous molecular and biological markers on lung cancer tissues and exfoliated cancer cells have been investigated *(2)*. However, evaluation of most of these transcripts, also known as biomarkers, often requires invasive procedures, such as bronchoscopy, to obtain tumor specimens. Obtaining tumor tissue may not always be logistically possible, thus preventing serial assessment during cancer progression or treatment.

The finding that tumors are capable of shedding nucleic acids (DNA or RNA) into the blood stream, which can be recovered from both serum and plasma and used as surrogate source of tumor DNA, has opened new areas in diagnosis and prognosis. Lung cancer develops through a multistage process with increasing genomic instabilities such as microsatellite alterations, loss of heterozygosity, and allelic shifts on chromosomal loci 3p, 9p, and 17p *(3–6)*; epigenetic changes, such as promoter hypermethylation of tumor suppressor genes *(7, 8)*; variations in the K-*ras (9)* and *p53 (10)* genes; and inactivation of the fragile histidine triad (*FHIT*) gene *(5)*. Detection of these changes in circulating cell-free DNA in lung cancer

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patients and chronic smokers has been proposed by several authors as a potential diagnostic tool (Table 1). It is believed that plasma/serum DNA is of tumor origin because the genetic alterations are similar to those found in the corresponding primary tumors *(11–13)*. Thus, quantification of cell-free DNA in plasma/serum and characterization of specific molecular changes could be very useful in the management and screening of lung cancer.

We conducted a systematic review of published original research reports investigating the role of free circulating DNA as a diagnostic and prognostic marker in lung cancer.

Literature Review

The clinical studies included in this review were identified through a literature search conducted through the MEDLINE database. The reference terms used for the search were "circulating DNA" and "lung cancer". In the MEDLINE database, the search was limited to human studies published in English. In addition, journal articles cited in the primary-search original reports and review articles were collected and added to the review. We also reviewed the abstracts presented at the annual meetings of the American Society of Medical Oncology during the past 3 years. Data were extracted from the reports, excluding studies that investigated tumor tissue, circulating RNA, occult/disseminated tumor cell DNA, and pleural/bronchial lavage fluid DNA; those with a sample size <20; and those evaluating malignancies other than lung cancer.

Mechanisms of Release of Cell-Free DNA into Circulation

Circulating extracellular DNA can be found in healthy persons and persons with nonmalignant diseases, including systemic lupus erythematosus, rheumatoid arthritis, pulmonary embolism, or myocardial infarction *(14–16)*, various malignancies, including small cell lung cancer (SCLC)[3] and non–small cell lung cancer (NSCLC) *(12, 13, 17)*. In addition, trauma *(18)* and therapeutic procedures *(19, 20)* may also lead to the release of free DNA into the circulation. The actual origin remains unknown, however. Considerable controversy exists concerning the reference concentrations of DNA in healthy controls, with reported values ranging from barely detectable concentrations *(20, 21)* to a few micrograms per liter *(22)*. Most of the authors of the published reports, however, agree that DNA concentrations are higher in persons with various malignant and nonmalignant disorders than in healthy controls. In healthy persons, it can be presumed that circulating DNA originates from lymphocytes or other nucleated cells, but its origin in malignancies is still unknown. Many mechanisms have been postulated that might explain the release of DNA into the circulation by tumor–host or tumor–virus interactions.

One of the mechanisms may involve lysis of cancer cells shed into the circulation by micrometastases. Such cells can be detected by various techniques, such as immunocytology *(23)*, flow cytometry *(24)*, and PCR-based *(25, 26)* methods using antibodies or probes specific to oncogenes, gene products, or characteristic intermediate filaments of epithelial cells, e.g., cytokeratin *(27)*. These techniques apply to one particular type of cancer, and sensitivity can be as high as 1 cancer cell in 10^7 peripheral blood mononuclear cells *(27)*. The number of circulating tumor cells, however, does not correlate with the total amount of free DNA. Sorenson et al. *(28)* and Chen et al. *(29)* calculated the amount of DNA and the number of cancer cells in plasma of cancer patients. For the amount of DNA they found in plasma, there would need to be 1000 to 10 000 cancer cells/mL, which is far more than the numbers of cells ever isolated by current techniques.

Cell necrosis may be another possible mechanism because higher amounts of DNA have been found in the plasma of patients with large or advanced/metastatic tumors *(30–37)*. It was noted, however, that after radiation therapy, which is presumed to induce cell death/necrosis, there was initial decrease rather than increase in circulating DNA in 40% of patients *(38)*. On the other hand, it is equally probable that cell arrest caused by radiotherapy reduced DNA release. It has been found that the activities of DNase I and II, which degrade DNA, are lower in malignant diseases *(39)*. Inhibitors of DNase have been detected in both tumors *(40)* and healthy cells such as thrombocytes *(41, 42)*.

Apoptosis has also been advanced as a possible source of circulating cell-free DNA on the basis that circulating DNA often gives a ladder pattern on electrophoresis similar to that shown by apoptotic cells *(31, 33, 43)*. However, it should be noted that apoptosis is a mechanism supposedly lost by proliferating cancer cells, and great efforts are required to restore programmed cell death in malignant cells. Moreover, the paradigm of apoptosis implies that epithelial cells and macrophages clear DNA-containing apoptotic bodies in situ without generating any inflammatory response *(44, 45)*.

Spontaneous, active release of DNA by tumors is another possibility *(46–51)*. The possibility that proliferating cancer cells release DNA into the circulation cannot be ignored because activated lymphocytes have been found to release DNA under in vitro conditions *(47)*. This could explain the presence of very low concentrations of cell-free DNA in small populations of cancer patients. In these cases, the cancer may have been relatively quiescent at the time of specimen collection. The decrease in free DNA concentrations after radiotherapy, as reported be Leon et al. *(38)*, may be attributable to arrest of cellular proliferation by radiation. To explore the potential mech-

[3] Nonstandard abbreviations: SCLC, small cell lung cancer; NSCLC, non–small cell lung cancer; and NSE, neuron-specific enolase; and hTERT, human telomerase catalytic subunit.

anisms of DNA release into the bloodstream, Sozzi et al. *(52)* evaluated necrosis, angiogenesis, and proliferation features in primary tumor samples. They found a significant association between plasma DNA concentration and microvessel density, suggesting a link with tumor angiogenic status. However, further work is needed to evaluate the possible origin of circulating DNA.

Genetic Changes in Circulating DNA vs Tumor DNA

The comparison of genetic alterations in plasma DNA and paired tumor DNA has led to different arguments regarding the heterogeneity of the tumors and the physiopathologic mechanisms of liberation of circulating DNA. After finding a significant correlation between plasma DNA concentrations and neuron-specific enolase (NSE) concentrations in SCLC, Fournie et al. *(33)* concluded that plasma DNA possibly originated from tumors because NSE has been regarded to originate from cancerous cells *(53)*, an observation that was experimentally supported in nude mice bearing human tumors *(33)*. The presence of genetic alterations in plasma DNA similar to those found in tumor DNA also supports the tumor origin *(11–13)*. It may be possible that this tumor-derived plasma/serum DNA might behave as an "infectious particle" or an "oncovirus" [many reports have indicated the presence of oncogenes in plasma/serum DNA (Table 1)] that transfects either nearby cells or cells of other organs to establish cell-free DNA-mediated metastases or secondary primary cancer.

In a study by Beau-Faller et al. *(54)*, 85% (17 of 20) lung cancer patients presenting with at least 1 allelic imbalance in tumor DNA also had at least 1 allelic imbalance in paired plasma DNA, but not necessarily at the same locus or on the same allele. These results demonstrate

Table 1. Summary of qualitative studies evaluating serum/plasma markers in lung cancer patients and controls.

Reference	Marker(s)	Source of DNA	No. of patients	Positive cases		Controls		Control population
				n	%	n	% positive	
(13)	Methylation of p16, DAPK,[a] GSTpi, and O6MGMT	Serum	22	11	50	0		
(70)	Methylation of p16, MGMT, DAPK, RASSF1A, and RAR-β	Serum	91	45	49.5	0		
(71)	Methylation of p16	Plasma	105	77	73.3	0		
(72)	Methylation of p16	Plasma	137	103	75.2	0		
(73)	Methylation of APC	Serum/Plasma	89	42	47	50	0	Healthy controls
(74)	Methylation of TMS1	Serum	50	17	34	0		
	RASSF1A		50	17	34	0		
	DAPK		50	20	40	0		
(75)	Methylation of p16	Plasma	35	12	34	15	0	Healthy controls
(76)	Methylation of APC,	Plasma	43	7	16.3	0		
	p16, and	43	6	14				
	MGMT/DAPK/GSTpi	43	NS	1–2				
(74)	K-*ras* variations	Serum	50	12	24	0		
(75)	K-*ras* variations	Plasma	35	0	0	0		
(81)	K-*ras* variations	Plasma	25	5	20	0		
(82)	K-*ras* variations	Plasma	48	17	35.4	40	30	Not given
(83)	β-Tubulin variations	Serum	131	55	42	112	0	Healthy controls
(11)	MSA-3 markers	Plasma	21	15	71	0		
(12)	MSA-2 markers	Plasma	87	35	40	14	0	Patients with carcinoids or other benign diseases
(54)	MSA-12 markers	Plasma	34	30	88	20	0	
(55)	MSA-3 markers	Plasma	35	25	71	0		
(56)	MSA-3 markers	Plasma	43	14	33	10	0	Healthy controls
(67)	MSA-5 markers	Plasma	38	9	24	43	0	Healthy controls
(75)	MSA-5 markers	Plasma	34	11	32	0		
(76)	MSA-16 markers	Plasma	32	22	69	0		
(84)	MSA-4 markers	Serum	22	6	28	0		
(85)	MSA-3 markers	Serum	28	17	61	31	0	Healthy controls
(86)	MSA-4 markers	Plasma	29	12	41	0		
(87)	*p53* and *FHIT* mutations and MAs on 3p loci	Plasma	64	33	51.6	0		

[a] DAPK, death-associated protein kinase; GSTpi, glutathione *S*-transferase π; O6MGMT, O^6-methylguanine methyltransferase; RASSF1A, ras association domain family 1A gene; RAR-β, retinoic acid receptor-β; APC, adenomatous polyposis coli; TMS1, target of methylation inducing silencing; FHIT, fragile histidine triad; MA, microsatellite alteration.

that genetic changes detected in plasma DNA are generally representative of the genetic change observed in paired abnormal biopsy specimens. However, different microsatellite alterations in serum/plasma vs primary tumor DNA have also been reported, notably in SCLC *(11, 12, 17, 55, 56)*. The discordant findings in tumor vs plasma DNA can be explained based on the presumption that different subpopulations of tumor clones with different genetic alterations might have differential access to the blood stream based on the physiologic properties of tumors, such as metastasis, angiogenesis, and capacity to cause thrombosis or necrosis.

Quantification of Circulating Cell-Free DNA

Many studies have addressed the use of serum/plasma DNA for disease management. In terms of DNA quantification, however, there is no standard. In the past, colorimetric or fluorometric assays used reagents such as diphenylamine, which when added to the patient's plasma/serum produced a color change, the degree of which correlated with the DNA concentration *(57–59)*. The poor specificity and sensitivity of the reagents and their side reactions with other components in samples has limited their use in quantitative analyses *(60, 61)*. Other assays, such as hemagglutinin inhibition, complement fixation, and diffusion in agarose, also did not provide advantage in terms of sensitivity *(20–22, 59, 62)*. With the more sensitive RNA-DNA hybridization, RIA, and counterimmunoelectrophoresis assays, nanogram amounts of circulating DNA can be quantified *(19, 63, 64)*. With real-time PCR *(18, 52, 65)* and PicoGreen double-stranded DNA quantification assays *(54, 66)*, it is now possible to quantify picogram amounts of circulating DNA.

The reported mean circulating DNA concentrations in patients with lung cancer vs controls and the effect of therapy during follow-up are summarized in Table 2. As evident in Table 2, significantly higher DNA concentrations can be detected in the serum/plasma of lung cancer patients compared with healthy controls or patients with benign diseases. However, in one study, DNA concentrations were higher in patients with benign lung diseases than in patients with lung cancer *(18)*, and this issue must be resolved. In addition, significantly higher DNA concentrations have been reported in the serum of patients with metastatic disease [mean (SE), 209 (39) μg/L] than in patients with nonmetastatic disease [100 (30) μg/L] *(38)*. Dot-hybridization experiments revealed that plasma DNA was increased above the cutoff value in 71% of the lung cancer patients, in 37% of the patients with benign pulmonary disease, and in none of the healthy controls *(32)*.

In terms of DNA quantification, existing studies vary with regard to the techniques used and the standardization and analysis procedures, thus preventing comparison of data across studies and estimation of the real prognostic potential of this approach. For example, in one study, the mean DNA concentration in lung cancer patients was 8-fold higher than in controls *(52)* but was 13-fold lower than the concentrations reported previously by the same group *(67)*. However, this difference was because the PCR methods used in the second report measured only amplifiable DNA *(52)*, whereas the methods used in the original report (spectrophotometer) could detect all DNA fragments *(67)*.

Defining DNA Cutoff Values: Effect of Therapy

To achieve maximum specificity and sensitivity, it is necessary to have a DNA concentration that does not overlap with the concentrations in control groups. Ranges of cutoff values have therefore been established, and 2 cutoff DNA values, one at which sensitivity was highest and another at which specificity was highest, are given in Table 3. From Table 3 it is clear that explicit cutoff values for DNA concentrations cannot be established at present because most of the published studies differ in the assays used. Three studies used real-time PCR for defining explicit DNA cutoff values *(52, 65, 68)*, but all used different genes for the amplification, e.g., human telomerase catalytic subunit (*hTERT*) *(52)*, immunoglobulin heavy chain consensus sequence *(65)*, and human β-actin gene *(68)*. It was found that higher cutoff values increased the specificity of the assay but at the cost of sensitivity and vice versa. In a study by Leon et al. *(38)*, 61% of lung cancer patients had higher circulating DNA concentrations [above the cutoff value of 50 μg/L; mean (SE), 164 (44) μg/L]; DNA concentrations decreased in 75% of these patients after therapy. The DNA concentration in the follow-up plasma samples (mean, 34 μg/L) was significantly lower than before surgery (mean, 345 μg/L) and was comparable to the concentration detectable in the control group *(67)*. Total DNA was increased in patients with untreated cancer and in those with disease recurrence, with a sensitivity of 75% and specificity of 86% *(67)*. From these studies it can be concluded that no explicit cutoff has been established that can serve as a valuable tool for the diagnosis and follow-up of individuals.

Correlation of Circulating DNA Concentrations with Various Prognostic Factors and Survival

No prognostic factor has been consistently reported to be associated with circulating DNA concentrations. Plasma DNA concentrations do not uniformly correlate with disease stage *(54, 66)*. Similarly, no correlation has been established with histologic subtypes. Xie et al. *(66)* reported higher amounts of circulating DNA in NSCLC compared with SCLC, results in contrast to those reported by Beau-Faller et al. *(54)*.

Disparity also exists with regard to variables such as clinical staging. In studies by Fournie et al. *(33)* and Xie et al. *(66)*, plasma DNA was highest in patients with stage IV disease, whereas in other studies there was no such association *(38, 52, 54, 65, 67, 68)*. An association with age was reported in one study *(52)* but not in the other studies *(38, 54, 65–68)*. No significant correlation was observed between plasma DNA concentrations and smoking inten-

Table 2. Quantitative studies on circulating DNA in plasma/serum of lung cancer patients.

Reference	Assay	Source of DNA	No. of patients	Mean DNA concentration, μg/L		Clinical correlation	Follow-up DNA concentration, μg/L	Control population
				Controls	Cancer			
(33)	Nick translation DNA labeling	Plasma	68	10	30	Plasma DNA concentration correlated with NSE, LD,[a] and survival	ND	Healthy individuals
(38)	RIA	Serum	33	13	164	No correlation with size, type, and location of tumor; higher DNA concentrations in metastatic disease	After radiotherapy, DNA concentrations decreased in 75% and increased in 25%	Healthy controls
(52)	Real-time PCR (gene: *hTERT*)	Plasma	100	3.1	24.3	Associated with age, microvessel density, and high risk of NSCLC; no correlation with smoking intensity or duration, tumor stage/type, and necrosis	8.4	Age-, sex-, and smoking-matched controls
(54)	Fluorescence assay using PicoGreen-dsDNA quantification reagents	Plasma	34	78	157	No correlation with tumor stage or type and prognosis in terms of patient's outcome in terms of overall survival and time to progression	ND	Patients with benign diseases
(65)	Real-time PCR (gene: immunoglobulin heavy chain-consensus sequence)	Serum	185	12.6	39.6	Plasma DNA concentrations correlated with LD, advanced tumor stage, and poor survival; serum DNA concentrations associated with leukocyte counts	In patients with tumor progression, plasma DNA increased, but not serum DNA	Healthy controls
		Plasma	185	1.8	3.7			
(18)	Fluorescence assay using SYBR Green II and Hoechst 33258.	Plasma	26	38	77.6	ND	ND	Healthy controls
(66)	Fluorescence assay using PicoGreen-dsDNA quantification reagents	Plasma	67	11.6	110.7	No correlation with tumor type or stage except stage IV.	ND	Healthy controls
(67)	DNA dipstick method	Plasma	84	18	318	No correlation of age, tumor type, or stage and prognosis in terms of overall or relapse-free survival	34	Healthy controls
(68)	Real-time PCR (gene: β-actin)	Plasma	25	10.6	14.6	No correlation with tumor stage or type	ND	Healthy controls
(76)	Quantitative RT-PCR (gene: β-actin)	Plasma	43	ND	5.55	Total DNA was detectable in all patients	Mean change in DNA was −2.5 μg/L for PR and +22.5 μg/L for PD/SD after radiology	NA
(88)	Quantitative RT-PCR (gene: β-actin)	Serum	100	ND	Stage III, 21.1; stage IV, 18.03	*hTERT* concentration was independent predictive variable for time to progression and overall survival	No effect of therapy on DNA concentrations	NA

[a] LD, lactate dehydrogenase; ND, not determined; dsDNA, double-stranded DNA; RT-PCR, reverse transcription-PCR; PR, partial response; PD, progressive disease; SD, stable disease; NA, not applicable.

Table 3. Performance evaluation of plasma DNA quantification as a screening tool to differentiate patients with lung cancer from controls.

Reference	Assay used	Cutoff DNA concentration, μg/L	Sensitivity, %	Specificity, %
(38)	RIA	>50	61	ND[a]
(52)	Real-time PCR (target gene: *hTERT*)	≥04	97	60
		≥25	46	99
(65)	Real-time PCR (target gene: immunoglobulin heavy chain consensus sequence)	>50	ND	98
(66)	PicoGreen assay	≥17.7	98	50
		≥256.1	25	100
(67)	DNA dipstick method	0–5	87	47
		26–125	54	100
(68)	Real-time PCR (target gene: β-actin)	14	48	100

[a] ND, not determined.

sity and duration and other features, such as necrosis, lymphoid infiltration, and growth patterns *(52)*. Serum DNA concentrations strongly correlated with patient leukocyte counts *(52)* (total neutrophil counts, not lymphocytes), an observation also found in leukemic patients *(69)*.

There are conflicting reports correlating the concentration of circulating DNA with survival. Some authors have reported no correlation between plasma DNA concentrations and relapse-free or overall survival *(54, 67)*, whereas other authors reported an association of plasma DNA with survival, lactate dehydrogenase *(33, 65)*, and NSE *(33)* for a mixed group of SCLC and NSCLC patients *(33)*, and for NSCLC patients only *(65)*. Gautschi et al. *(65)* reported that tumor progression after chemotherapy was significantly associated with increasing plasma DNA concentrations. Generally, when the treatment was beneficial, as determined by a decrease in tumor size or reduction in pain, a decrease in serum DNA concentrations was observed *(38, 65)*, but not in plasma DNA *(65)*. Conversely, when the treatment was unsuccessful, serum DNA concentrations tended to remain unchanged or even increased *(38)*. Plasma DNA increased but remained below the cutoff value in most patients with disease progression *(65)*. Overall, the median DNA concentration during follow-up (8.4 μg/L) showed a clear trend toward decreases, suggesting that quantification of plasma DNA might represent a novel approach to monitor surgical procedures or to assess the efficacy of chemo-/radiotherapy *(52)*.

Qualitative Studies on Circulating DNA

Given the diversity of findings in quantitative studies—e.g., the actual origin of circulating DNA, the cause of increased DNA concentrations in lung cancer and some benign nonmalignant diseases compared with healthy controls, and the various conflicting reports of their correlation with various clinicopathologic and histologic features—where do we go from here? One step is to use serum/plasma DNA to study various genetic changes that occur during the progression of lung cancer.

There have been many studies regarding the epigenetic changes, microsatellite instabilities, and variations in serum/plasma DNA (Table 1). However, except for a few, most of the studies did not involve controls; therefore, comparison of the data across cases and controls is not possible. Methylation-specific PCR techniques have been used in at least 8 studies *(13, 70–76)* to quantify the methylation of the promoter regions of several oncogenes. Liu et al. *(72)* reported the highest frequency (75.2%) by detecting promoter hypermethylation of p16 gene. In all other methylation studies, the frequency of cancer detection varied from 1%–2% to 73.3% (Table 1). Microsatellite alterations, such as loss of heterozygosity and allelic shifts, have also been reported in at least 12 studies (Table 1). Many of these studies used 2 to 5 markers, except for 2 studies, which used 12 *(54)* and 16 *(77)* markers. The best sensitivity (88%) for detecting microsatellite alterations in plasma DNA was reported by Beau-Faller et al. *(54)*, who used a series of 12 markers. The sensitivity of microsatellite alteration detection in serum/plasma DNA varied from 24% to 71% in other studies. However, none of the studies reported microsatellite alterations in the healthy control populations.

The discrepancies in sensitivities across the studies could be attributable to technical differences: the methods for DNA isolation, the sequences and choice of primers, the microsatellite markers themselves, the amplification process, and radioactive vs fluorescent analysis. In addition, there was no uniformity in the selection of markers used in these studies. Choice of microsatellite alterations could influence the results. Microsatellite alterations in lung cancer are restricted to single loci and are not a generalized phenomenon as seen in hereditary nonpolyposis colorectal cancer *(78)*. Hence, to detect microsatellite alterations in lung cancer, one must select markers that display a high polymorphism rate and are changed in most, if not all, of the tumor cells. A lower plasma frequency of allelic imbalance may be observed for larger-sized gene products because plasma DNA is highly fragmented and alterations may therefore be detected more easily if the sequence to be amplified is smaller than

200 bp (79). To compare the results obtained in different laboratories, it is necessary to standardize the methods used and to select an optimized panel of genetic markers.

The choice of cohort studied (stage of disease and/or histologic subtype) could also influence the results. Higher frequencies and incidences of plasma DNA alterations have been noted in more advanced stages of lung cancer, similar to studies of kidney cancer (80). Plasma allelic imbalance frequencies at 3p (particularly at 3p21) and *TP53* have been more frequently described for SCLC, whereas plasma allelic imbalances at 20q were observed more frequently in NSCLC (especially in metastatic disease) (54). On the basis of specific differential findings, a routine panel of marker has been suggested that included markers at 3p and 17p13 for SCLC and at 5q, 9p, 9q, and 20q for NSCLC (54).

At least 4 studies investigated K-*ra*s variations in serum or plasma (74, 75, 81). Ramirez et al. (74) reported K-*ras* variations in 24% of cases and Kimura et al. (81) reported variations in 20% of cases, whereas Bearzatto et al. (75) found no K-*ras* variations among 35 cases (Table 1). Betta et al. (82) reported K-*ras* variations among 30% of the controls. For oncogene or tumor suppressor gene variation screenings, enrichment techniques that increase the sensitivity of molecular analysis are available, but they are rather expensive and time-consuming and require the previous knowledge of variations in tumor specimens. Thus, they do not appear to be useful in screening programs (including individuals with high risk, e.g., heavy smokers) in the absence of tumor DNA.

Chemoresistance Markers in Circulating DNA

Resistance to available chemotherapy drugs and targeted therapies is one of the important obstacles to effective treatment. Drug resistance may result from multiple mechanisms, such as changes in cellular drug uptake, metabolic drug deactivation, structural changes in the drug target, or changes in other cellular components that interact with the target. Some examples of molecular mechanisms of drug resistance relevant to lung cancer include (*a*) cisplatin resistance associated with the nucleotide excision repair pathway; (*b*) chemoresistance associated with overexpression of nuclear factor-κB, a transcription factor; (*c*) paclitaxel resistance associated with the presence of β-tubulin variations; (*d*) interference in gemcitabine metabolism resulting from chromosomal deletions, e.g., 11p15.5, that affect the genes involved in deoxyribonucleotide synthesis, such as ribonucleotide reductase; (*e*) gefitinib resistance associated with epidermal growth factor receptor variations; and (*f*) analysis of genes involved in cell cycle regulation and development as possible predictive markers of chemosensitivity.

All of these genetic changes potentially can be studied in circulating DNA. Rosell et al. (83) analyzed the presence of β-tubulin variations in serum DNA from 131 NSCLC patients matched with serum DNA obtained from 112 healthy individuals. β-Tubulin variations were detected in serum DNA from 42% of the patients but in none of the healthy controls. Thus, the study of serum or plasma DNA has opened new roads for translational research and new strategies for identifying genetic changes that might predict chemoresistance or chemosensitivity.

Future Prospects

The presence of circulating tumor DNA in the serum/plasma of lung cancer patients has sparked great interest because conventional diagnostic tests tend to be imperfect and more invasive, posing logistic difficulties for serial tumor sampling. Less-invasive techniques, such as blood tests, are attractive for screening, diagnosis, prognosis, surveillance for occult disease progression, identification of potential therapeutic targets, monitoring of tumor responses, and evaluation of disease pathophysiology and biology. However, several steps are needed before such tests can be used.

The tremendous disparities among the quantitative and qualitative reports on circulating DNA have introduced considerable confusion. These disparities may be attributable to (*a*) lack of adequate standards to evaluate DNA in plasma/serum, (*b*) different methods used for measuring DNA, and (*c*) differences in sample-processing techniques. It is evident that any future application of plasma or serum DNA analysis for diagnostic or prognostic purposes will depend on the reproducibility and reliability of results, both of which require the optimization and equivalence of procedures.

There appear to be 2 major clinical avenues to pursue for the use of circulating DNA markers. The first is the use of circulating DNA to monitor for disease recurrence or to establish disease-free status. However, it is clear that large prospective studies with long follow-up times are essential to evaluate the circulating DNA markers and eventually integrate them into the clinical setting. The second is to validate the use of simple blood tests that can detect early-stage lung cancers, which are more amenable to surgical resection and treatment. New techniques that combine automated DNA extraction and amplification with multiplexed fluorescent PCR and multichannel analysis of circulating DNA, which is a favorite cancer research target, hold potential for use as population-based screening tools for lung cancer. Plasma/serum DNA concentrations could possibly help identify high-risk individuals for chemoprevention trials and could be tested as potential intermediate biomarkers of the efficacy of intervention.

Conclusions

The findings from molecular qualitative studies on serum/plasma DNA suggest that it may be possible to develop a simple blood test that could improve the diagnosis and monitoring of lung cancer. The low sensitivity of such tests could be compensated when combined with spiral computed tomography or positron emission tomography. A panel of tumor-specific genetic markers in circulating

DNA with high sensitivity and specificity may be used: (*a*) as a complementary noninvasive assay for early diagnosis and screening of high-risk individuals, such as chronic smokers; (*b*) to measure intermediate endpoints of efficacy in chemoprevention trials; (*c*) for prognostic or diagnostic purposes (e.g., staging and determination of tumor load); and (*d*) as surrogate markers for treatment response or failure. However, further work is clearly needed to refine existing techniques for sample collection and processing, to improve semiquantitative and quantitative PCR assays, and to identify additional, more specific, and more sensitive serum- and plasma-based biomarkers.

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