Biomarkers of Gene Expression: Growth Factors and Oncoproteins

Paul W. Brandt-Rauf

Division of Environmental Health Sciences, Columbia University School of Public Health, New York, New York

This article reviews the literature on the application of methods for the detection of growth factors, oncogene proteins, and tumor-suppressor gene proteins in the blood of humans with cancer or who are at risk for the development of cancer. The research summarized here suggests that many of these biomarker assays can be used to distinguish between diseased and nondiseased states and in some instances may be able to predict susceptibility for future disease. Thus, these biomarkers could be valuable tools for monitoring at-risk populations for purposes of disease prevention and control. — Environ Health Perspect 105(Suppl 4):807–816 (1997)

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Introduction

The development of many cancers and some other environmentally related diseases is believed to be associated with the aberrant expression of genes that encode proteins involved in cellular growth signal transduction, including growth factors and the protein products of oncogenes and tumor-suppressor genes. This aberrant expression can involve a quantitative difference from normal (i.e., overexpression of the wild-type protein) and/or a qualitative difference (i.e., expression of a mutant form of the protein). The detection of increased expression of these proteins or expression of mutant forms of these proteins therefore represents a category of potential biomarkers with which to study susceptibility to the development of disease, particularly cancer.

Numerous studies have documented this aberrant expression of growth factors and oncoproteins in diseased tissue compared to normal tissue. However, the techniques are somewhat complicated, and, in most cases, it would be difficult to obtain tissue samples for routine screening of at-risk populations. Fortunately, in many instances it is apparent that these proteins gain access to the extracellular environment and are thus detectable in easily obtainable biological fluids such as serum or plasma by techniques that are relatively simple, convenient, and easily standardized. Thus, this review will focus on the assay of growth factors and oncoproteins in extracellular fluids, particularly serum and plasma, by techniques that could readily be applied to study at-risk populations in developing as well as developed countries.

Growth Factors

Since growth factors are normally actively secreted from cells, they represent logical targets for detection in blood during disease development. Several studies have demonstrated differences in blood levels of growth factors between cancer patients and controls.

Platelet-derived Growth Factor

Platelet-derived growth factor (PDGF), which functions as a dimer of A and B chains (PDGF-B is encoded by the sis oncogene), has been examined in the blood of various cancer patients. Plasma PDGF-B chain levels were determined by enzyme-linked immunosorbent assay (ELISA) in 131 patients with cancers of various types (including carcinomas, sarcomas, and lymphomas) and 72 noncancer controls (1). Levels were greater than the highest control value of 0.69 ng/ml in 19 (15%) of the cases, although, based on other markers, in only 5 (4%) of the cases was the tumor felt to be the source of the growth factor. Plasma PDGF levels have also been determined by radioimmunoassay (RIA) in 58 breast cancer patients and 9 normal female controls (2). All of the controls were below the lower limit of detection of the assay (1.56 fmol/100 μl), but 20 of 17 (12%) stage II cancer patients had detectable levels and 13 of 41 (32%) stage IV cancer patients had elevated levels (defined as more than twice the lower limit of detection of the assay). Patients with elevated PDGF had a significantly greater degree of metastatic involvement and a significantly shorter survival time. Elevated levels of PDGF-related proteins have also been detected by immunoblotting in the urine of cancer patients (3).

Transforming Growth Factor α

Transforming growth factor α (TGFα) is a 50-amino acid, single-chain polypeptide growth factor, and its proliferative effect is mediated through the epidermal growth factor receptor. TGFα, as determined by ELISA in plasma, was initially reported to be elevated in 71 patients with solid tumors (mean ± SD = 346 ± 155 pg/ml) compared to 66 controls (mean ± SD = 187 ± 29 pg/ml); but the differences were not statistically significant (4). In another study, TGFα levels in pooled plasma samples determined by ELISA were 0.051 ng/ml in cancer patients (stomach, colon, liver) compared to 0.028 ng/ml in 15 healthy volunteers (5). TGFα levels have also been measured by RIA in the serum of 83 breast cancer patients and 74 healthy controls (6). Among the controls, 24 had nondetectable serum TGFα (< 100 pg/ml) and 50 (67%) had detectable levels that ranged from 120 to 207 pg/ml (mean ± SD = 147 ± 18 pg/ml). All of the cancer cases had detectable serum TGFα levels that ranged from 210 to 740 pg/ml (mean ± SD = 353 ± 98 pg/ml), and the difference in the means between cases and controls was statistically significant (p < 0.001). The same RIA was also used to
TGFβ1, TGFβ2, and TGFβ3 levels were measured by ELISA in the plasma of 28 breast cancer patients and 42 normal controls (13). Control values ranged from 2 to 12 ng/ml (mean ± SD = 4.1 ± 2.0 ng/ml), and 2 of the cancer patients (7%) were found to have elevated levels (defined as more than 2 SD above the control mean). TGFβ2 was detectable in 2 of the cancer patients also, but not in any of the controls.

Basic Fibroblast Growth Factor

Basic fibroblast growth factor (bFGF) is a single-chain polypeptide composed of 146 amino acids. Basic fibroblast growth factor was initially reported to be detectable by ELISA in the serum of renal cell carcinoma patients (14). Follow-up study showed detectable levels (>30 pg/ml) in serum in 28 of 52 (54%) renal cell carcinoma patients, 8 of 30 (27%) urothelial cancer patients, 2 of 7 (29%) prostatic cancer patients and 0 of 12 (0%) testicular cancer patients (15). Five of 8 patients with renal cell carcinoma who underwent selective renal venous sampling prior to nephrectomy had increased serum bFGF in the renal vein from the affected kidney. Within 2 weeks of resection, serum bFGF became undetectable, suggesting that the tumors were the source of the increased bFGF in serum in those cases. Using an enhanced chemiluminescence enzyme immunoassay, elevated serum bFGF levels (defined as more than 3 SD above the normal mean, i.e., >22.1 pg/ml) were found in 0 of 25 (0%) controls, 0 of 16 (0%) stomach cancers, 0 of 6 (0%) bladder cancers, 2 of 23 (9%) liver cancers, 9 of 16 (56%) brain cancers, 21 of 30 (70%) renal cancers, and 19 of 24 (79%) lung cancers (16). Mean serum bFGF levels determined by fluorometric enzyme immunoassay were also significantly elevated in cases of esophageal, stomach, colon, liver, breast cancer (p < 0.001) and pancreas cancer (p < 0.01). Relatively high levels were found in 1 patient with adenocarcinoma of the lung, 4 of 6 cases of lymphoma, and several cases of gall bladder and thyroid cancer (17). A follow-up study in breast cancer patients showed serum bFGF levels higher than those of any normal controls in 25 of 35 (71%) stage I patients, 10 of 13 (77%) stage II patients and 5 of 5 (100%) stage III patients (18). Furthermore, in all patients undergoing surgical resection, serum levels were statistically significantly lower after surgery than before, suggesting that the tumors were the source of the increased bFGF in serum in those cases.

Levels of bFGF have also been reported to be elevated in the serum in other breast cancer patients (19), in the plasma of patients with multiple endocrine neoplasia type I (20) and of patients with B-cell chronic lymphocytic leukemia (21), and in the serum of patients with cervical cancer (22). In the latter study, four of the 20 patients relapsed after complete remission and two of these had a continuous increase in serum bFGF levels before the clinical detection of relapse with a mean lead time of 4 months, suggesting that serum bFGF may be useful for the early detection of recurrences and possibly primary tumors.

Other

Plasma or serum levels of several other growth factors have been reported in various cancers. Epidermal growth factor (EGF) is elevated in the serum of some patients with stomach cancer (23), cancer of the tongue (24), and ovarian cancer (25), but unchanged or decreased in other cancers (26–28). Insulinlike growth factors, (IGF) have been reported to be elevated in the plasma of some breast cancer patients (29) and ovarian cancer patients (25), but not in other cancers (30,31). Elevated serum levels of hepatocyte growth factor (HGF) have been reported in hepatocellular carcinoma as well as in nonmalignant liver diseases (32). In addition, many types of growth factors have been identified in other biological fluids, including urine (33), effusions (34), cyst aspirates (35,36), and bronchoalveolar lavage (37).

Growth factors probably also play important roles in non-cancer proliferative diseases, such as fibroproliferative disorders; and serum or plasma levels have been determined in some cases. For example, elevated TGFβ levels in plasma after induction chemotherapy are predictive of liver and lung fibrosis in patients receiving bone marrow transplantation for cancer (positive predictive value > 0.90) (38). Elevated serum levels of TGFβ have also been reported more frequently in firefighters, particularly those with a history of asbestos exposure and chest radiographs consistent with asbestosis exposure, compared to matched controls (39). Similarly, elevated serum levels of PDGF were reported in 25 of 45 (56%) pneumoconiosis patients, and elevated levels were statistically significantly more frequent in radiographically advanced cases compared to less advanced cases (p = 0.016) with a tendency for these cases to have progression of their fibrotic disease over the course
of the study (40). Finally, in atherogenesis, high levels of total growth factor activity in plasma, 20% of which is attributable to PDGF, has been found to be significantly correlated with progression \((r = 0.42, p < 0.05)\) and severity \((r = 0.52, p < 0.01)\) of coronary atherosclerosis with elevated PDGF levels specifically correlated with the number and severity of stenoses \((r = 0.40, p < 0.05)\) (41).

**Oncoproteins**

**Growth Factor Receptors**

Transmembrane growth factor receptors are frequently overexpressed in human malignancies. Overexpression is accompanied in many instances by cleavage of the extracellular domain (ECD) of the receptor with its accumulation in the extracellular environment. Thus, detection of increased amounts of the ECD of these receptors in blood is a potential biomarker of cancer development.

Many studies have examined the ECD of the p185 transmembrane growth factor receptor (encoded by the c-erbB-2 oncogene) in the blood of cancer patients. An initial study reported elevated serum erbB-2 ECD levels by ELISA (40- to 190-fold higher than controls) in 3 of 12 (25%) breast cancer patients compared to 35 controls (42). Two of the cases with serum elevation also had increased tissue expression, suggesting that the tumors were the source of the serum proteins. Another study reported elevated serum erbB-2 ECD by ELISA (defined as greater than 2 SD above the mean of normals) in 3 of 42 (7%) normal women, 5 of 33 (15%) women with untreated primary breast cancer, and 24 of 105 (33%) women with metastatic breast cancer (43). In another study, elevated serum erbB-2 ECD by ELISA was detected in 0 of 30 (0%) cases of benign breast disease, 2 of 64 (3%) cases of stage I/II primary breast cancer, 5 of 17 (29%) cases of stage III/IV primary breast cancer, 3 of 12 (33%) cases of locally recurrent breast cancer, 26 of 51 (51%) cases of recurrent metastatic disease, but 0 of 57 (0%) cases with no evidence of recurrence; in addition, there was a close association between serum elevation and tissue overexpression, and in several cases changes in serum levels reflected the clinical status of disease (44, 45). Similarly, 12 of 53 (23%) patients with metastatic or locally advanced breast cancer were reported to have elevated levels of serum erbB-2 ECD by RIA compared to 0 of 69 (0%) controls; in two cases, changes in serum levels correlated with disease status during therapy (46).

Another study reported elevated serum erbB-2 ECD levels by ELISA in 0 of 19 (0%) controls, 0 of 35 (0%) patients without metastatic disease following removal of the primary breast tumor, and 9 of 26 (35%) patients with residual metastatic disease, 3 of whom had correspondingly elevated tumor tissue expression (47).

Pupa et al. reported elevated serum erbB-2 ECD levels by RIA in 0 of 50 (0%) healthy controls and 0 of 25 (0%) breast cancer cases with stage I/II disease compared to 6 of 40 (15%) cases with stage III/IV disease, and the correlation between tumor overexpression and serum elevation was statistically significant \((p < 0.01)\) (48). Kath et al. (49, 50) reported elevated serum erbB-2 ECD levels by ELISA in 26 of 61 (43%) patients with metastatic breast cancer, and there was reasonably good correlation between serum and tissue levels of expression and with clinical course of disease (49, 50). Breuer et al. found 1 of 25 (4%) matched controls had elevated serum erbB-2 ECD by ELISA compared to 9 of 36 (25%) cases with newly diagnosed primary breast cancer \((p = 0.03)\); 2 cases with elevated serum levels had tumor tissue overexpression and two cases with elevated preoperative levels had normal postoperative levels (51, 52). In addition, in this study, there were 7 cases of in situ carcinoma without invasion, and 3 of these (43%) had elevated serum ECD levels, suggesting that this may be a biomarker of early malignant disease in certain cases of breast cancer. Many additional studies on elevated serum erbB-2 ECD in breast cancer have been reported (53-59). In the most recent study, elevated serum erbB-2 ECD was found in 3 of 66 (5%) controls, 0 of 12 (0%) cases of benign breast disease, 1 of 13 (8%) preoperative breast cancer cases, 2 of 62 (3%) postoperative cases without recurrent disease, and 55 of 93 (59%) cases with recurrent disease; elevated serum level was statistically significantly associated with protein overexpression in the tumor \((p = 0.044)\) (60). An inducible immune response to c-erbB-2 oncoprotein (production of antibodies against the protein from circulating lymphocytes isolated and transformed with Epstein-Barr virus) has also been identified in breast cancer patients (61).

Elevated serum erbB-2 ECD levels have also been identified in other cancers. In ovarian cancer, elevated levels were reported in 7 of 48 (15%) patients with a correlation between serum and tissue overexpression (62). Elevated serum erbB-2 ECD levels were reported in ovarian cancers as well as colorectal, pancreatic, prostate, and liver cancers (63). Similarly, patients with gastric cancer have been found to have elevated levels with a good correlation between serum and tissue overexpression (64-66). In another study, levels in the plasma of colonic adenoma patients were statistically significantly elevated compared to controls; patients with large adenomas had higher levels than patients with small adenomas (67). In addition, the average serum erbB-2 ECD level in male Taiwanese who subsequently developed hepatocellular carcinoma was statistically significantly elevated compared to matched controls who did not develop cancer, and increasing levels showed a significant linear trend in relation to the subsequent development of cancer, with those individuals with elevated levels averaging over 2 years between the time of serum collection and the diagnosis of disease (68, 69). These results support the hypothesis that serum erbB-2 ECD may be a biomarker of early malignant disease in some cases. Elevated serum erbB-2 ECD levels have also been described in patients with lung cancer (70). In another study, levels measured in multiple banked serum samples from 11 pneumoconiosis patients who subsequently developed lung cancer were found to be statistically significantly elevated compared to controls, and in 4 of the cases, levels were elevated prior to the diagnosis of disease with a lead time averaging 35 months (71). This similarly supports the potential utility of this biomarker for the early diagnosis of malignant disease.

Recently, the ECD of the epidermal growth factor receptor (EGFR, encoded by the c-erbB-1 oncogene) has also been identified in cancer patients. The EGFR ECD was quantitated by ELISA in the banked serum samples from 38 asbestosis cases who subsequently developed cancer, 72 asbestosis controls without cancer matched for age, sex, race, smoking, and asbestos exposure, and 20 nonasbestosis, noncancer controls matched for age, sex, race, and smoking (72, 73). The mean serum level for the EGFR ECD in the cancer cases \((mean ± SD = 636 ± 299 fm/ml)\) was statistically significantly elevated \((p < 0.05)\) in comparison to the mean level in the asbestosis controls \((mean ± SD = 546 ± 147 fm/ml)\) or the nonasbestosis controls \((336 ± 228 fm/ml)\). Seven cancer cases had elevated serum levels prior to the time of disease diagnosis with an average lead time.
of 5.1 years, suggesting that this too may be a potential biomarker of early malignant disease. Serum EGFR ECD levels have also been found to be statistically significantly elevated among 22 former uranium miners with lung cancer compared to 7 healthy controls \((p = 0.007)\) \((74)\). The ECD of EGFR has also been measured in the urine of cancer patients. Elevated levels were detected in 15 of 42 (36%) squamous cell carcinoma patients compared to 8 of 50 (16%) non-squamous cell carcinoma patients and 3 of 50 (6%) non-cancer controls, statistically significant differences \((p<0.03)\) \((75)\).

**Other Oncogene Proteins**

Although the mechanism by which they gain access to the extracellular environment is uncertain, several nonreceptor oncogene proteins, including the membrane-associated \(\text{G}\) proteins and the nuclear DNA-binding proteins, have been identified in blood.

The \(\text{ras}\) oncogene encodes a 21-kDa membrane-associated \(\text{G}\) protein \((p21)\) involved in growth signal transduction from transmembrane growth factor receptors to cytoplasmic kinases and ultimately to the nucleus. The \(\text{ras}\) oncogene is activated in carcinogenesis either by overexpression of \(p21\) or by expression of point-mutated forms of \(p21\). The \(p21\) \(\text{ras}\)-related protein was elevated in serum by ELISA in 5 of 34 (15%) patients with early stage malignancies and 26 of 59 (44%) patients with advanced malignancies compared to 1 of 58 (2%) controls, with the highest levels in patients with lymphoma, breast, and urogenital malignancies \((76, 77)\). In another study, increased serum \(p21\) by ELISA was found in 3 of 13 (23%) patients with stomach cancer compared to 0 of 3 (0%) normal controls, although no increases were noted in 29 other patients with different cancers \((78)\). Elevated serum \(p21\) has also been identified in individuals at risk for the development of cancer due to workplace carcinogen exposures \((79–81)\). In one of these cases, an individual with elevated serum \(p21\) by immunoblotting 18 months later developed a premalignant colonic lesion, and once the lesion was removed the individual’s serum \(p21\) returned to normal \((82)\). This suggests that the tumor was the source for the elevated protein in serum and that this biomarker may be detectable prior to the identification of clinical disease. Elevated serum \(p21\) has also been identified in lung cancer patients \((83, 84)\). In another study of multipleanked serum samples from 46 pneumoconiosis patients, elevated serum \(p21\) levels were demonstrated by immunoblotting in 7 of 18 (39%) patients who developed cancer (5 of which were lung cancers) compared to 2 of 28 (7%) patients who did not develop cancer, a statistically significant difference \((p = 0.012)\) \((40)\). In addition, 6 of the 7 cancer cases had elevated serum \(p21\) prior to the time of clinical diagnosis \((average = 16.3 months)\), again suggesting that this may be a biomarker of early malignant disease in certain cases. In another study, elevated serum \(p21\) by immunoblotting was found in up to 54 of 80 (67.5%) cases of various cancers (including lung, colon, breast, prostate, and liver) compared to 30 of 188 (15.9%) non-cancer controls, a statistically significant difference \((85)\). In a recent study, elevated plasma \(p21\) by immunoblotting was found in 4 of 47 (8.5%) controls, 10 of 54 (18.5%) colon adenoma cases, and 9 of 22 (40.9%) colon carcinoma cases, statistically significant difference between cancers and controls \((p = 0.003)\) \((86)\). In this study, plasma \(p21\) overexpression increased with increasing size of adenoma and increasing stage of carcinoma, and there was a statistically significant correlation between overexpression in the plasma and in the corresponding tumor tissue.

Mutant \(\text{ras}\) \(p21\) protein has also been detected in blood. Asp 13 mutant \(p21\) was studied by immunoblotting in the serum of patients with angiosarcoma of the liver (ASL) and in individuals with heavy vinyl chloride \((\text{VC})\) exposure who are at risk for the development of ASL \((87, 88)\). In this study, four of five (80%) cases of ASL were found to contain the mutant \(\text{ras}\) gene in their tumor tissue and to express the corresponding mutant \(p21\) in their tumor tissue and in their serum, whereas the one case of ASL without the mutation and a case of hepatocellular carcinoma without the mutation did not have detectable mutant \(p21\) in their tumor tissue or in their serum. In one of the ASL cases with multiple serum samples over time, levels of mutant \(p21\) appeared to correlate with the clinical status of disease. In addition, 8 of 9 (89%) individuals with VC-associated, nonmalignant angiomatous lesions of the liver and 22 of 45 (49%) individuals with heavy VC exposure but no detectable liver lesions also had mutant \(p21\) in their serum compared to 0 of 28 (0%) controls matched for age, sex, and race. Stratification of this cohort by years of VC exposure showed a significant linear trend \((p < 10^{-3})\) for the occurrence of the serum mutant \(p21\) with increasing duration of exposure, and, since increased exposure is associated with increased cancer risk, this suggests that serum mutant \(p21\) may be a biomarker of early carcinogenic change in some cases.

Other biomarkers of \(\text{ras}\) gene mutation have also been identified in blood. For example, the identification of mutant \(\text{ras}\) genes by polymerase chain reaction and direct sequencing of DNA isolated from the serum or plasma of three patients with pancreatic cancer has been described \((89)\). Furthermore, circulating antibodies directed against Asp 12 mutant \(p21\) have been detected by ELISA in 51 of 160 (32%) colon cancer patients compared to 1 of 40 (2.5%) normal controls \((90)\). Elevated levels of the \(\text{ras}\) \(p21\) protein as well as mutant forms have also been identified by immunoblotting in the urine of cancer patients \((3, 91)\).

The \(\text{myc}\) oncogene encodes a 64-kDa nuclear protein that forms a heterodimer with the \(\text{max}\) oncogene protein and binds to specific DNA sequences, resulting in the transcription of other genes involved in controlling the cell cycle. Overexpression of the \(\text{myc}\) oncoprotein has been noted in many human malignancies, and increased levels have been detected in blood. For example, increased \(\text{myc}\)-related protein has been identified in the serum by immunoblotting in 51 patients with a wide variety of solid tumors compared to 16 controls with nonmalignant disease and 17 healthy controls \((92, 93)\). In 12 of the cancer cases, localization of the production of the \(\text{myc}\) protein to the tumor was demonstrated \emph{in vivo} by radioimmunoscintigraphy, and serial measurements in patients with resected colorectal carcinomas showed a gradual return to normal levels following surgery. In another study, increased levels of \(\text{myc}\) protein were identified by immunoblotting in 7 of 36 (19%) breast cancer cases compared to 0 of 25 (0%) matched controls, statistically significant difference \((p = 0.02)\) \((51, 94)\). In two cases, increased \(\text{myc}\) protein was also identified in the tumor tissue, and in one case, increased serum levels returned to normal following the removal of the tumor. In addition, one case of intraductal carcinoma without evidence of invasion was serum-positive for \(\text{myc}\) protein, suggesting that this may be a biomarker of early malignant disease in some cases. Serum antibodies to the \(\text{myc}\) protein have also been identified in cancer patients. For
example, circulating myc antibodies were first described in 4 of 6 (67%) colon cancers, 12 of 125 (10%) breast cancers, 1 of 2 (50%) osteosarcomas, 1 of 9 (11%) ovarian cancers, and 3 cancers of unknown origin (95). A follow-up study demonstrated myc antibodies in serum of 25 of 44 (57%) cases of colorectal cancer compared to 8 of 46 (17%) normal controls (p = 0.001) (96). Antibodies to the myc protein have also been described in the sera of patients with myeloid leukemia and lymphoma, including Burkitt’s lymphoma (97). Serum antibodies to other oncogene proteins have also been identified in cancer patients (98).

Tumor Suppressor Gene Proteins

The most frequent site for mutations in human cancers is the tumor suppressor gene encoding p53, a 53-kDa nuclear protein. The effect of these mutations is to cause loss of the normal growth inhibitory function of p53 with a concomitant accumulation of the mutant proteins in the transformed cells due to the considerably increased half-lives of mutant p53s. Accumulations of mutant p53 have been frequently identified by immunohistochemistry in human tumors and in some cases lead to accumulations in the extracellular environment resulting in potential biomarkers in the blood.

Increased levels of mutant p53 in serum determined by ELISA (greater than 0.3 ng/ml, the upper limit of 100 normals) was first reported in 11 of 54 (20%) patients with hepatocellular carcinoma as well as 30% of patients with cirrhosis, a group known to be at increased risk for the development of hepatocellular carcinoma (99). Elevated serum mutant p53 levels determined by ELISA have also been reported in 5 of 60 (8%) breast cancer patients, with levels decreasing following surgical resection of the tumors, although tissue immunohistochemistry for p53 correlated poorly with serum levels (100). In another study, elevated serum mutant p53 by ELISA was found in 15 of 82 (18%) breast cancer patients compared to 0 of 20 (0%) normals (101). Elevated serum mutant p53 levels determined by ELISA and immunoblotting were also found in 3 of 23 (13%) lung cancer patients compared to 0 of 23 (0%) controls matched for age, sex, and race and 2 of 58 (3%) unmatched controls, and increased tissue p53 and/or the presence of p53 gene mutations were found in the 3 serum-positive cancer cases (102). In a larger study of lung cancer patients, elevated levels of mutant p53 were detected by ELISA in the serum of 17 of 50 (34%) non-small cell lung cancer patients compared to 0 of 15 (0%) controls, and the levels of p53 protein accumulation in the tumor tissue by immunohistochemistry were found to be strongly correlated with the levels of p53 in the serum (p = 0.007) (103). Elevated levels of mutant p53 have also been reported in the plasma of 21 of 65 (32%) patients with non-Hodgkin’s lymphoma (104) and in the serum of 6 of 33 (18%) patients with Hodgkin’s lymphoma (105). In another study, elevated total serum p53 protein (greater than 10 controls) was reported in 6 of 16 (38%) patients with colon cancer and 18 of 28 (64%) patients with colon carcinomas (106). In another study of colon neoplasms, plasma levels of mutant p53 were found to be statistically significantly elevated among 54 cases of colon adenomas (mean = 0.44 ng/ml) and 22 cases of colon carcinomas (mean = 0.55 ng/ml) compared to 47 individuals with negative colonoscopic examinations (mean = 0.12 ng/ml) (p < 0.02), and plasma levels tended to increase with increasing adenoma size and increasing carcinoma stage (107). Total serum p53 levels have also been found to be statistically significantly elevated among 22 former uranium miners with lung cancer compared to 7 healthy controls (p = 0.003) (74). In a study of banked serum samples from asbestos patients, elevated total and mutant serum p53 was found in up to 6 of 32 (19%) patients who subsequently developed cancer compared to 2 of 36 (6%) asbestos patients without cancer and 1 of 10 (10%) nonasbestos controls; in 1 serum-positive case of lung cancer, elevated p53 levels were found in the tumor tissue, and in several of the cases elevated levels were present in serum years prior to the clinical diagnosis of disease (8,108). Serum mutant p53 has also been examined in vinyl chloride-exposed workers with and without angiosarcomas of the liver. Two cases of ASL known to contain p53 gene mutations were found to have elevated serum mutant p53 by ELISA compared to 2 cases of ASL and the one case of hepatocellular carcinoma known not to contain p53 gene mutations. In addition, 3 of 19 (16%) VC-exposed workers without liver lesions were also serum-positive for mutant p53 compared to 0 of 5 (0%) matched unexposed controls (109). These results, together with those on banked serum samples, suggest that serum p53 may be a biomarker of early malignant change in some cases. Other studies, however, have failed to detect elevated serum p53 in cancer patients (110).

Serum antibodies against p53 have also been reported in patients with several types of cancer. p53 antibodies in serum were first reported in 14 of 155 (9%) breast cancer patients compared to 0 of 164 (0%) controls (111). Froment et al. reported p53 antibodies in the sera of 14 of 119 (12%) children with various types of cancer, including 6 of 28 (21%) cases of B-cell lymphoma, compared to 1 of 88 (1%) controls (112). p53 antibodies have been found in the sera of 6 of 46 (13%) patients with lung cancer compared to 0 of 51 (0%) controls, and in this study, all antibody-positive cases had p53 gene missense mutations and increased p53 protein in their tumors (113). Similarly, p53 antibodies have been found in the sera of 7 of 60 (11%) patients with breast cancer compared to 0 of 15 (0%) controls, and all 7 positive cases had p53 gene mutations and increased p53 protein in their tumors (114). Schlichtholz et al. also reported p53 antibodies in the sera of 15 of 100 (15%) patients with breast cancer (115). p53 antibodies were reported in the serum in 9 of 175 (5%) patients with various cancers (including colon, breast, lung, and ovary) compared to 0 of 22 (0%) controls (116). In another study, p53 antibodies were identified in serum in 20 of 80 (25%) patients with hepatocellular carcinoma compared to 0 of 67 (0%) controls (117). Antibodies to p53 have also been found in the serum in 12 of 93 (13%) breast cancer patients, 2 of 83 (2%) prostate cancer patients, 4 of 108 (4%) thyroid cancer patients, 10 of 42 (24%) lung cancer patients, 8 of 29 (28%) bladder cancer patients, 4 of 88 (5%) leukemia patients, 14 of 73 (19%) pancreas cancer patients, and 1 patient each with ovarian cancer, hepatoma, and kidney cancer (118). In another study, p53 antibodies were found in the sera of 10 of 42 (24%) patients with lung carcinoma compared to 2 of 58 (3%) controls without malignant respiratory diseases, but one of the positive controls was found to have a tracheal chondroma and the other was diagnosed with lung cancer 4 months later, suggesting that p53 antibodies may be an early marker of neoplasia in certain cases (119). Angelopoulou and Diamandis reported p53 antibodies in serum determined by two different methods in 3 of 105 (3%) breast cancer patients, 10 of 22 (14%) ovarian cancer patients, 11 of 77 (14%) colon cancer patients, and 2 of 46 (4%) pancreas cancer.
patients (120). Mudenda et al. reported p53 antibodies in the serum of 48 of 182 (26%) breast cancer patients compared to 1 of 76 (1.3%) normal controls, and there was a significant correlation between serum positivity and increased p53 protein in the tumor; in addition, 8 of 23 (35%) patients with ductal carcinoma in situ were positive for p53 antibodies, again suggesting that this may be an early marker of disease in some cases (121). In a large recent study of p53 antibodies in serum in 1392 patients with various malignancies determined by two methods, the highest prevalence of antibodies was found in ovarian and colon cancers (15%), lung cancers (8%) and breast cancers (5%) with lower prevalences in other malignancies (<4%) and controls (1–2%) (122). In a study of vinyl chloride-exposed workers, 9 patients with ASLs were found to have p53 antibodies in their serum, and in 3 cases these were detectable prior to the clinical detection of disease (average = 8 years), once again supporting the hypothesis that this may be an early biomarker of disease in certain cases (123).

Finally, another recent study reported p53 antibodies in serum by two different ELISAs in 16 of 136 (11.8%) lung cancer patients compared to 0 of 52 (0%) patients with nonmalignant pulmonary disease; however, although 47 of the tumors contained p53 gene mutations, only 7 of those were antibody positive, and of 32 tumors with accumulations of p53 protein, only 5 were antibody positive (124).

Summary

The research summarized here suggests that the expression of genes involved in growth-signal transduction, as determined by detection of growth factors and oncoproteins in easily accessible extracellular fluids, may represent convenient biomarkers for monitoring related disease processes, particularly cancer (for summary see Table 1). In some cases, these biomarkers not only have shown the ability to distinguish between diseased and nondiseased states but also have suggested the possibility of detection early in the disease process indicating susceptibility to future development of disease. By and large, however, these studies have focused on acquired defects in the expression of these growth factors and oncoproteins rather than on inherited defects, which would be more typical for other susceptibility biomarkers. Nevertheless, the potential exists for the application of these assays for detecting related inherited defects, for example, in individuals with inherited mutations in p53 in the Li-Fraumeni syndrome and in other elevated risk circumstances. For instance, a recent study identified increased levels of the extracellular domain of the erbB-2 oncoprotein in healthy asymptomatic women to be associated with other established risk factors for breast cancer, including a family history of a grandmother with breast cancer, among others (52). These assays may thus be able to detect both acquired and inherited susceptibility states. In addition, many of the assays involved are relatively simple and straightforward immunologic analysis techniques amenable for routine use in any hospital laboratory, including those in developing countries. However, additional study will be necessary before these assays can be considered for such routine use. Further work will be required in terms of standardization of assay techniques, correlation of blood and tissue expression, the kinetics of release from cells and elimination from the blood, assay reproducibility, stability of stored samples, better definition

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**Table 1. Summary of studies of growth factors and oncoproteins as extracellular biomarkers of gene expression.**

| Biomarker | Method of analysis | Associated diseases | References |
|-----------|--------------------|---------------------|------------|
| **Growth factors** | | | |
| PDGF | ELISA, RIA, and immunoblot in plasma, serum, urine, or bronchoalveolar lavage | Breast cancer, various other carcinomas, sarcomas, lymphomas, lung fibrosis, pneumoconiosis, atherosclerosis | (1-3,37,40,41) |
| TGFα | ELISA and RIA in plasma, serum, cyst aspirates, or effusions | Breast cancer, various other carcinomas, pneumoconiosis | (4-8,34,35) |
| TGFβ | ELISA, immunoblot and activity assay in plasma, serum, or cyst aspirates | Liver cancer, bladder cancer, breast cancer, leukemia, liver and lung fibrosis, pneumoconiosis | (9-13,35,38,39) |
| bFGF | ELISA and other ELA in serum or plasma | Kidney cancer, breast cancer, various other cancers | (14-22) |
| EGF | RIA in plasma, serum, urine, or cyst aspirates | Stomach cancer, ovarian cancer, various other cancers | (23-28,33,36) |
| IGF | ELISA and RIA in plasma or serum | Breast cancer, ovarian cancer, hepatitis, cirrhosis | (25-29-31) |
| HGF | ELISA in serum | Liver cancer, hepatitis, cirrhosis | (32) |
| **Oncoproteins** | | | |
| erbB-2 | ELISA and RIA in serum or plasma | Breast cancer, ovarian cancer, liver cancer, lung cancer, various other cancers | (42-60, 62-71) |
| erbB-2Ab | Immunoprecipitation of supernatant of cultured lymphocytes | Breast cancer | (61) |
| EGRF | ELISA in serum or urine | Lung cancer, various other carcinomas | (72-75) |
| ras | ELISA, immunoblot, and PCR in serum, plasma, or urine | Lung cancer, colon cancer, angiosarcoma of liver, various other cancer | (3,4,10,79-89,91) |
| ras Ab | ELISA in serum | Colon cancer | (90) |
| myc | Immunoblot in serum | Lung cancer, colon cancer, breast cancer | (52,92-94) |
| myc Ab | Immunoblot in serum | Colon cancer, breast cancer, lymphoma, various other cancers | (95-97) |
| myb Ab | Immunoblot in serum | Various cancers | (90) |
| p53 | ELISA, immunoblot, and immunofluorometry in serum or plasma | Lung cancer, breast cancer, colon cancer, liver cancer, lymphoma | (8,74,99-110) |
| p53 Ab | ELISA and immunoblot in serum | Lung cancer, breast cancer, colon cancer, liver cancer, leukemia, lymphoma, various other cancers | (111-124) |
of significant blood levels in terms of pathophysiologic importance and in relation to the range of normal values determined in large populations, definition of potential confounding factors, and assessment of sensitivity, specificity, and predictive value of the tests. Recent advances suggest that detection sensitivity for many of these assays can be greatly improved in the near future, which could help to resolve some of these issues. For example, the combination of current immunochromatographic techniques with DNA amplification technology based on the polymerase chain reaction (immuno-PCR) offers the prospect of increasing sensitivity by 100,000-fold or more (125). Ultimately, highly sensitive, specific, standardized assays for growth factors and oncoproteins (alone or in combinations) could be valuable tools for monitoring at-risk populations for purposes of disease prevention and control.

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