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Review article

Oncogene proteins as biomarkers in the molecular epidemiology of occupational carcinogenesis

The example of the *ras* oncogene-encoded p21 protein*

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Summary. The use of oncogene proteins as biomarkers offers a new approach to the molecular epidemiologic evaluation of occupational carcinogenesis. The *ras* oncogene-encoded p21 protein represents a prototype for this type of study, since it is known to be activated by common occupational carcinogens, is frequently found in human tumors of occupational concern, and, at least in certain instances, appears to be expressed relatively early in the disease process, allowing the possibility of early detection and intervention. Herein, we review our experience with the use of immunologic detection of p21 in cohorts with cancer or at risk for the development of cancer due to their occupational exposures. The results suggest that p21 (particularly when used with other oncoproteins and biomarkers such as PAH-DNA adducts) will indeed be a useful addition to the growing armamentarium of molecular epidemiologic biomarkers in the study of occupational carcinogenic mechanisms and in the detection and prevention of occupational cancers.

Key words: Oncogenes – Biomarkers – Molecular epidemiology – Occupational cancer – *Ras* gene

Introduction

Despite major advances in recent decades, occupational cancer remains a significant problem, and the prevention of all occupational cancer remains a theoretically obtainable but as yet elusive goal (Landrigan and Markowitz 1989). One obstacle to approaching this goal has been the difficulty of traditional epidemiologic approaches in providing precise estimates of risks associated with chronic low doses of carcinogens as encountered in the workplace. In these situations, the lack of accurate data concerning the real extent of exposure and its biologic consequences is a serious handicap acting to dilute true associations that therefore go unrecognized.

For this reason, there has been considerable interest in the development of sensitive and specific biomarkers that can better identify and quantify carcinogen exposure or measure the consequences of such exposure (Santella et al. 1987). This molecular epidemiologic approach is an attempt to bridge the gap between traditional environmental hygiene measurements of ambient carcinogen exposure and traditional epidemiologic assessment of resultant cancers (Perera and Weinstein 1982). Besides improving our understanding of carcinogenic pathways and mechanisms and allowing refinement of the dose-response relationships in epidemiologic evaluations, it is hoped that such biomarkers will ultimately have predictive value in pinpointing those individuals in an exposed worker population who are at greatest risk for the development of malignancy at a sufficiently early stage that the process may be successfully aborted. Biomarkers thus provide multiple opportunities for approaching the goal of prevention of all occupational cancer (Hulka and Wilcosky 1988; Cullen 1989).

Molecular epidemiology and occupational cancer biomarkers

Biomarkers of occupational carcinogenesis have been studied at three levels: markers of internal dose, markers of biologically effective dose, and markers of preclinical response (Brandt-Rauf 1988). Markers of internal dose usually consist of determinations of the amount of a carcinogen or its active metabolite that has actually been absorbed into the body and is present in biological fluids such as serum or urine. However, since measure-

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ment of a carcinogen's concentration in body fluids cannot necessarily indicate the degree of its delivery to and interaction with critical sites in target tissues, alternative approaches based on the biologically effective dose have been developed. As the dose of carcinogen required for the initiation of carcinogenesis has been assumed to be the amount that binds to the DNA of the target cell, various indices of formation of DNA-carcinogen adducts have been used to evaluate the biologically effective dose. However, even excess formation of DNA adducts does not guarantee that the target cell will become committed to the cancer pathway and result in clinically significant disease.

The clinically significant result of the formation of DNA-carcinogen adducts is presumed to be the irreversible alteration of the cellular genome, for example, by producing point mutations or chromosome breaks and rearrangements. Thus, chromosomal aberrations, sister chromatid exchanges, or micronuclei formation could potentially serve as useful markers of significant preclinical response to occupational carcinogen exposure. However, as knowledge of the molecular biology of carcinogenesis has advanced, it has become increasingly clear that in many instances these markers of chromosomal damage are merely gross indices of alterations in a particularly small subset of the human genome, namely, the oncogenes. Thus, it has been suggested that oncogenes may be valuable biomarkers in the molecular epidemiologic study of occupational and environmental carcinogenesis (Brandt-Rauf 1988; Taylor 1989).

Oncogenes and oncogene proteins

It now seems likely that all eukaryotic cells contain a subset of genes that are critical to normal cellular growth and development (proto-oncogenes) but can become inappropriately activated, yielding genes (oncogenes) with the ability to produce malignant transformation of cells. In fact, there are probably several dozen proto-oncogenes in normal human cells that can be activated to oncogenes by a number of mechanisms, including point mutation and overexpression (Pimentel 1986).

Point mutation, which could be produced by exposure to a variety of occupational carcinogens, has been shown to be important in the activation of the *ras* and *neu* (also known as *HER*-2 or *erb*B-2) oncogenes. The *neu* oncogene is frequently found in certain types of human carcinomas (e.g., breast cancers) and has been shown to be activated in animal models by nitrosourea-induced mutation. Resultant selected amino acid substitutions for Val 664 in the transmembrane domain of the *neu* oncogene-encoded protein product (p185) enable this protein to transform cells malignantly. The p185 protein is apparently a receptor for an as yet unidentified growth factor, and the result of the mutation is the production of a conformational change in the protein that can cause signal transduction in the absence of this external stimulus (Brandt-Rauf et al. 1989a, 1990a). However, the prototypical example of mutational oncogene activation is provided by the *ras* gene (Barbacid 1987; Balmain and Brown 1988).

The *ras* oncogene is frequently found in many different types of human cancers (including those of occupational concern such as lung cancer) and has been shown to be activated in in vitro or in vivo models by occupational carcinogens such as many of the polycyclic aromatic hydrocarbons (PAHs), nitrosoureas, and ionizing radiation; in fact, evidence suggests that different types of exposure produce different types of *ras* gene activation (Balmain and Brown 1988), an observation that may be of significance in attempts to link causal exposures to resultant disease. Selected amino acid substitutions at various positions (including Gly 12, Gly 13, Ala 59, Gln 61, Glu 63, and Asp 119) in the *ras* oncogene-encoded protein product (p21) enable this protein to transform cells malignantly. Once again, this is likely due to conformational changes in the p21 protein structure produced by the amino acid substitutions (Brandt-Rauf et al. 1985, 1988; Pincus and Brandt-Rauf 1985, 1986; Pincus et al. 1987).

For many oncogenes, overexpression of the normal proto-oncogene-encoded protein products seems to be sufficient to cause cell transformation. Such gene overexpression can occur due to gene amplification, gene deregulation due to the insertion of transcriptional regulatory sequences, or gene rearrangements. These changes may be produced by carcinogen-induced chromosomal breaks. An example of such proto-oncogene activation is provided by the *myc* gene, whose actively oncogenic version is found in Burkitt's lymphoma in humans. The gene becomes transposed from its usual position at the end of chromosome 8 to the ends of chromosomes 2, 14, or 22, where it can be juxtaposed to genes responsible for immunoglobulin synthesis, which are being transcribed at a high rate. Resultant overexpression of the *myc* gene results in cell transformation (Erikson et al. 1983).

The aforementioned *neu* and *ras* genes can also be activated by overexpression. In both cases, this may be due to the observation that even for the normal proto-oncogene-encoded proteins, a small proportion of molecules may exist in the same conformation assumed by the mutant transforming proteins. At normal levels of expression, the presence of this small number of molecules in a transforming conformation apparently has no physiologic effect. However, with overexpression of the proto-oncogene, a sufficient number of protein molecules in this minority-transforming conformation are generated to result in cell transformation (Brandt-Rauf et al. 1990a; Pincus and Brandt-Rauf 1986). Thus, gene overexpression is clearly another significant method of oncogene activation.

The *ras* gene and p21 protein

One of the best-studied oncogene systems is the *ras* gene family and its encoded p21 protein. Also, the *ras* gene may be the most relevant oncogene for occupational cancer studies. As noted above, this gene is known to be activated by common carcinogens of occupational concern. In addition, it is frequently found to be activated in human tumors of all different types, including those of occupational concern such as lung cancer.

For example, one recent study of lung cancer patients (Rodenhuis et al. 1987) revealed that 50% (5/10) of those with adenocarcinomas had a specific form of activated *ras* oncogene (K-*ras*). All five patients with *ras*-positive adenocarcinomas were heavy cigarette smokers, whereas two of the patients with *ras*-negative adenocarcinomas had never smoked and a third had stopped smoking 13 years prior to the development of his lung cancer, providing a possible link between exposure to smoking-related carcinogens such as PAHs and particular oncogene activation in lung cancer. Furthermore, two of the tumors with activated oncogenes measured < 2 cm and had not yet metastasized; thus, the authors concluded that *ras* gene activation may be an important early event in the pathogenesis of adenocarcinoma of the lung. This latter point is of obvious significance if *ras* oncogene activation is to be used as a suitable biomarker of occupational carcinogenesis, since it would allow early detection and prevention.

That *ras* gene activation may be an early event in the carcinogenic process is further supported by the finding that not only are activated *ras* genes frequently found in colonic carcinomas, but they also occur in common premalignant lesions such as colonic polyps (Spandidos and Kerr 1984). Thus, because *ras* can be activated by occupational carcinogens, is frequently found in human tumors of occupational concern, and can be activated at relatively early stages in the carcinogenic process, the *ras* gene system may serve as a prototype for oncogene biomarkers of occupational carcinogenesis.

Ras stands for rat sarcoma, the site where the oncogene was first identified. However, the *ras* gene is identical to a gene that was isolated in a human bladder-carcinoma cell line. It was shown that the latter gene could be transfected into cells in culture, producing cell transformation and that these cells would produce tumors when transplanted into animals. It has also been shown that mammalian *ras* genes encode a protein of 189 amino acids that has a molecular mass of 21 kDa and is hence designated as p21. The exact function of p21 is not clear. It is obviously important to cell division, since cells in culture that have had the gene deleted cannot divide completely. The p21 protein is contained in all eukaryatic cells bound to the inner surface of the plasma cell membrane by the carboxyl terminus. All p21 proteins bind guanosine triphosphate with high affinity and specificity. The normal protein contains guanosine triphosphatase activity that is about 1 order of magnitude greater than that of the activated protein due to the differential effect of binding to a GTPase-activating protein. This evidence suggests that p21 is probably a signal-transducing G-protein. Signal transduction may involve the phospholipase C-phosphatidylinositol pathway, but this remains unclear (Barbacid 1987; Balmain and Brown 1988). It is apparent that p21 interacts with several other intracellular proteins (Lee et al. 1989), and as these are further defined they may also possibly serve as useful biomarkers of cell transformation.

As with all genes, it is apparent that the transforming effects of the *ras* gene are directly exerted through its p21 protein product. This conclusion is supported by microinjection and cell fusion experiments (Stacy and Kung 1984; Feramisco et al. 1985; Lee et al. 1989). For example, when activated p21 protein is introduced into nontransformed cells in culture that contain the normal *ras* proto-oncogene, transient cell transformation is produced, with the cells gradually returning to phenotypic normality as the protein is degraded intracellularly. Furthermore, when monoclonal antibody directed against activated p21 protein is introduced into cells transformed by the *ras* oncogene, the cells temporarily revert to a normal phenotype as the antibody binds to the activated protein, negating its effect. Thus, it appears that activated *ras* genes can produce cell transformation, one of the critical steps in the multistage progression from normalcy to malignancy, and that this transforming effect is mediated by the encoded p21 protein product. Therefore, the p21 protein should serve as a useful biomarker in the study of *ras* oncogene-induced occupational carcinogenesis.

The p21 protein as a cancer biomarker

From the viewpoint of molecular epidemiology, it is noteworthy that *ras* gene activation in human tumors can easily be detected through the application of immunologic techniques using monoclonal antibodies that can identify increased amounts or mutant forms of the p21 protein in cells. This approach can thus be used to differentiate immunohistochemically between transformed cell lines or cancer tissue and nontransformed or normal cells (Brandt-Rauf and Pincus 1987). For example, antibodies to the *ras* gene product have been used to detect elevated levels or mutant forms in 11 of 23 primary human lung cancers (Kurzrock et al. 1986). Similar results have been obtained in studies of cancer of the colon, breast, and liver (Thor et al. 1984; Agnantis et al. 1986; Habib and Wood 1986). Furthermore, as noted above, *ras* gene activation may occur quite early in the carcinogenic process; thus, this approach may be applicable for the detection of lesions that are not yet malignant but have a high potential for becoming so.

Of course, the ability to discover oncogene protein products prior to frank malignancy would be of great value both for monitoring the carcinogenic process and for measuring the risk for cancer. Thus, the use of immunohistochemical techniques to detect p21 in tissue could have direct applicability to the screening of cohorts exposed to occupational carcinogens in instances where cellular materials are easily available. For example, sputum or urine cytology specimens could be stained with monoclonal antibodies so as to improve the sensitivity and specificity of these methods of early diagnosis of respiratory or genito-urinary malignancies, areas of critical concern in occupational carcinogenesis. We have successfully used this technique to detect cells expressing the *ras* gene product in an otherwise normal sputum cytology specimen from a smoking asbestos worker who would clearly be considered at risk for the development of lung cancer due to those exposures (Brandt-Rauf et al. 1990b).

However, since it is not usually feasible to obtain tissue for screening for occupational cancer, it would be most useful if oncogene products such as p21 protein could be detected in more easily accessible biological fluids. For this to be the case, the oncogene protein products must reach the extracellular environment. In certain instances such as those involving the growth factors TGF and the *sis* gene product PDGF, this is clearly the case since the proteins are actively secreted by cells (Pimentel 1986). However, even in the case of the p21 *ras* gene product, we found that viable transformed cells in culture shed significant amounts of the p21 protein into the extracellular environment, where it could be easily detected by immunologic techniques using monoclonal antibodies (see Fig. 1). Thus, p21 protein should be identifiable in such easily accessible biological fluids as urine and serum in in vivo situations where the *ras* gene is being expressed. This has been demonstrated in animal models. Cells in culture that have been transformed by the *ras* gene and are expressing p21 have been transplanted into nude mice, resulting in tumors, and p21 has been detected in the animals' sera using immunologic techniques (Carney, personal communication; Kakkanas and Spandidos 1990).

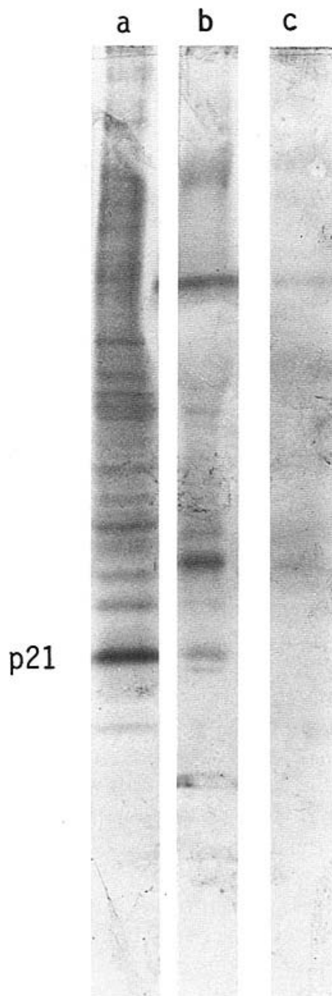

Fig. 1. Immunoblots using monoclonal antibody to the peptide sequence of the H-*ras* gene of: cell lysate of K22 rat liver epithelial cells transfected with T24 and overexpressing the H-*ras* gene *(a);* supernatant from the same cells *(b);* supernatant from nontransfected control cells *(c)*. Note the band for p21 protein in the cell lysate and supernatant of the cells expressing the *ras* gene, which is absent from the control supernatant

This approach has been applied to study the urine of human cancer patients. In this case, 5- to 20-fold increases in the levels of oncogene-related proteins (including the *ras* gene protein) were detected in the urine of 172 cancer patients (including cancers of occupational concern such as bladder, lung, and skin carcinomas), occurring in patterns that were different from those seen in normal, healthy controls (Niman et al. 1985). We have used this approach in the detection of oncogene proteins, including p21, in sera from individuals with cancer or at risk for the development of cancer due to their previous exposures to occupational carcinogens.

Although urine may be more easy to obtain for screening purposes, the advantage of serum over urine is that certain oncogene proteins may be too large to pass the glomerulus and would thus be missed in urine screening. Furthermore, the test requires only 0.1 ml of serum to screen for the presence of as many as 16 different types of oncogene activation, and this amount of serum is usually easily obtainable for screening studies. The test itself is an immunoblot that relies on electrophoresis of the serum sample followed by transfer to nitrocellulose, reaction with the monoclonal antibody directed against the amino acid sequence of the oncogene protein, and detection using a secondary antibody with a radioactive or enzymatic-colorimetric label; band intensity can be determined by serial dilution or quantitated using a densitometer (Brandt-Rauf and Niman 1988).

This approach appears to be highly sensitive, specific, and reproducible for the purposes of human studies (Brandt-Rauf 1990b). For example, sensitivity is demonstrated by the ability to detect < 1 ng of p21 protein in a group of serially diluted standard samples (see Fig. 2). Specificity is demonstrated by the ability to block the detection of p21 in a known positive sample by competitive binding through preincubation of the monoclonal antibody with p21 protein or its epitopic peptide sequence and by the failure to block such detection by preincubation with proteins or peptide sequences of other onco-

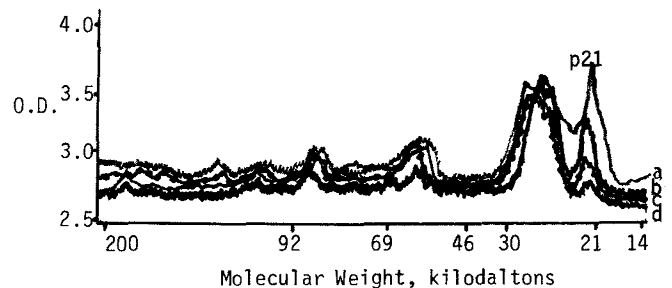

Fig. 2. Densitometer optical density tracings of immunoblots using monoclonal antibody to the peptide sequence of the H-*ras* gene of serially diluted standard samples of the p21 protein: 100 ng *(a);* 10 ng *(b);* 1 ng *(c);* 0.1 ng *(d)*. Note the peaks at 21 kDa in all samples

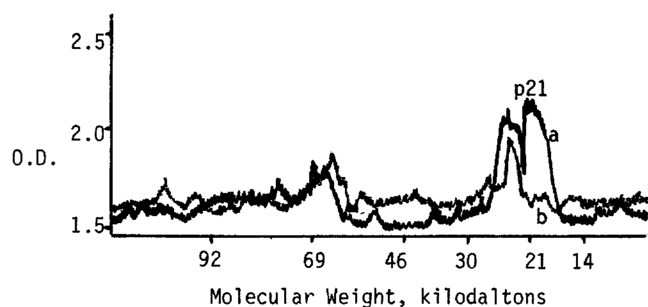

Fig. 3. Densitometer optical density tracings of immunoblots using monoclonal antibody to the peptide sequence of the H-*ras* gene of: a serum sample from an individual with tumor expressing the H-*ras* gene *(a)*; a serum sample from the same individual after preincubation of antibody with p21 protein or its epitopic peptide *(b)*. Note the peak at 21 kDa in the first sample, which is competitively blocked in the second sample

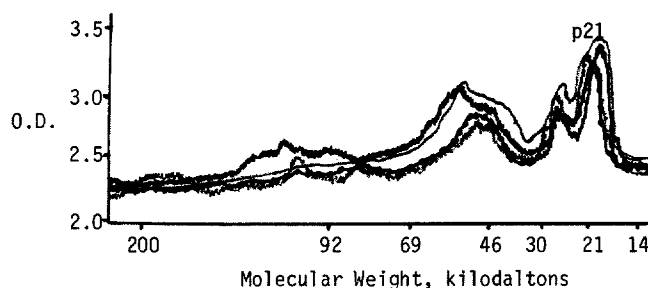

Fig. 4. Densitometer optical density tracings of immunoblots using monoclonal antibody to the peptide sequence of the H-*ras* gene of serum samples from an individual with tumor expressing the H-*ras* gene. The different tracings represent assays on different serum samples taken from the same individual at intervals of approximately 1 month. Note the reproducibility of the tracings over time

Table 1. Serum p21 protein and PAH-DNA adducts in lung cancer patients

	Patients (*n*)	Smokers (*n*)	Average PAH-DNA adducts (fmol/μg)[a]	Subjects positive for p21 (*n*)
Lung cancer patients	18	18	0.25	15
Controls	18	8	–	2[b]

[a] For lung cancer patients this represents the average for the three patients where levels were detectable; no determinations were made in the controls

[b] Both of these individuals were heavy cigarette smokers; all nonsmoking controls were negative; all positive lung cancer patients were current or ex-smokers as indicated

gene proteins (see Fig. 3). Reproducibility is demonstrated by the ability to reproduce the same immunoblot patterns on repeat assays of the same sample or repeat assays on samples taken from the same individual at different times (see Fig. 4).

Initially, this technique was applied to the detection of oncogene proteins in sera from lung cancer patients (see Table 1; Brandt-Rauf et al. 1990b). A total of 18 patients with histologically documented non-small-cell cancers of the lung were selected for study; all of these subjects were heavy current or former cigarette smokers (average, 52 pack-years). Blood from these patients was screened for the presence of PAH-DNA adducts in peripheral lymphocytes and for the presence of oncogene proteins including p21. In all, 3 individuals were positive for PAH-DNA adducts (average, 0.25 fmol/μg) and 15 were positive for p21 oncogene protein, including the 3 who were positive for PAH-DNA adducts. (A control population of 18 healthy subjects included 2 individuals who were positive for the *ras* gene protein. However, it was discovered that both of these individuals were heavy cigarette smokers; all nonsmoking controls were negative.) These results confirm that p21 protein can be detected in sera from individuals who have cancers associated with a known carcinogenic environmental exposure, in this case cigarette smoke.

In addition, six cancer patients (four with lung cancer and two with esophageal cancer) have been followed using serial oncogene protein assays during the course of their therapy (Perera et al. 1990). All six subjects were found to have p21 protein in their serum; four of them (two lung cancers and two esophageal cancers) showed no response to therapy and their pattern of p21 expression did not change over time. The two other lung cancer patients were believed to have achieved clinically complete response to therapy. However, their pattern of p21 expression also failed to evidence any improvement over time, suggesting that they still had a significant residual burden of tumor; within 2 years, both patients had suffered a relapse and succumbed to their disease. These results suggest that serum oncogene proteins may not only correlate with the clinical disease state but may also actually precede and, thus, predict the clinical recurrence of disease. Further support for this is provided by studies of urine screening for oncogene proteins. In one breast cancer patient who was in remission, recurrence of an abnormal oncogene protein pattern (including *ras*-related proteins) in her urine predated the reappearance of clinically detectable disease by several months (Niman et al. 1985).

These results suggest that it may be possible to detect p21 proteins in the sera of clinically healthy individuals who are at risk for the development of malignancy due to occupational or environmental exposures at a point in the disease process prior to the time of clinical presentation, i.e., serum oncogene protein expression may help to predict those individuals in an exposed cohort who will develop neoplastic disease. Thus, this technique has been applied to cohorts of workers who might be at elevated risk for cancer due to their workplace exposures to carcinogens.

A cohort of 18 Finnish foundry workers with known high ambient workplace exposures to PAHs, particularly benzo(a)pyrene (BP), who were historically presumed to be at high risk for the development of lung cancer due to their occupational exposure, were examined for PAH-DNA adducts and serum oncogene proteins including p21 (see Table 2; Brandt-Rauf et al. 1990c). Based on industrial hygiene sampling data and job de-

Table 2. Serum p21 protein and PAH-DNA adducts in foundry workers

Ambient exposure (μg BP/m^3)	Patients (n)	Smokers (n)	Average PHA-DNA adducts (fmol/μg)[a]	Subjects positive for p21 (n)
Exposed (> 0.05)	8	5	1.08	1[b]
Unexposed (< 0.05)	10	4	0.14	0

[a] For both groups this represents the average for those individuals whose levels were detectable; all 8 exposed workers had detectable values, but only 5 of the unexposed controls had detectable levels; for the exposed workers this represents levels after at least 6 weeks of exposure at work

[b] This individual was also a smoker, but it should be noted that the major contribution to body burden of PAHs in the exposed group is not due to smoking but to workplace exposure, which represents the equivalent of smoking several packs of cigarettes per day

scriptions, these individuals were grouped into the categories of exposed (>0.05 μg BP/m^3, 8 h TWA) and unexposed controls (Perera et al. 1988). PAH-DNA adducts in the peripheral lymphocytes of these workers were found to correlate with levels of ambient exposure (average PAH-DNA adducts, 1.08 fmol/μg in the exposed group vs 0.14 fmol/μg in the unexposed group). In addition, one individual in the exposed group was identified as being positive for the *ras* oncogene p21 protein product; no positive test for serum oncogene proteins was obtained in the unexposed controls. Based on the aforementioned findings in lung cancer patients, one can speculate that the foundry worker with p21-positive serum is the individual in this cohort who is most likely to develop subsequent malignancy. Further follow-up will be necessary to substantiate this hypothesis.

We have also used this approach to screen sera from a cohort of 16 hazardous-waste workers with histories of known multiple-carcinogen exposures (including polychlorinated biphenyls, asbestos, PAHs, chlorinated hydrocarbon solvents, and ionizing radiation) for oncogene proteins including p21 and compare them with an age/sex/race-matched cohort of 17 control hazardous-waste workers who were presumed to have minimal carcinogen exposure due to their use of appropriate work practices and personal protective equipment (see Table 3; Brandt-Rauf and Niman 1988; Brandt-Rauf et al. 1989b; Brandt-Rauf 1990a). Of the 16 exposed workers, 3 were found to be positive for p21. Only 2 of the 17 controls tested positive for p21, but both of these individuals were heavy cigarette smokers; all nonsmoking controls were negative.

Once again, it is tempting to speculate that the individuals with positive serum oncogene proteins are those most likely to develop subsequent malignancy. In fact, one individual in this cohort provides support for this premise. The worker with the worst history of occupational carcinogen exposure and the highest level of expression of the *ras* oncogene-encoded p21 protein remained clinically healthy for approximately 18 months after the initial testing. However, at that time he developed rectal bleeding and was found to have a premalignant colonic polyp; it was postulated that prior heavy exposure to asbestos and, possibly, other carcinogens may have contributed to his colonic neoplasia. At any rate, upon removal of the polyp, this individual's serum p21 pattern reverted to normal and he has since remained free of disease (see Fig. 5; Brandt-Rauf et al. 1990d). This case provides support for the concept that serum screening for oncogene proteins (particularly the *ras* gene p21 protein) may indeed be a useful tool for the early detection and prevention of cancers in individuals with carcinogen exposure.

Table 3. Serum p21 protein in hazardous-waste workers

	Patients (n)	Smokers (n)	Subjects positive for p21 (n)
Exposed	16	11	3
Controls	17	6	2[a]

[a] Both of these individuals were only trace-positive and both were heavy cigarette smokers; all nonsmoking controls were negative; two of the three positives in the exposed group were nonsmokers

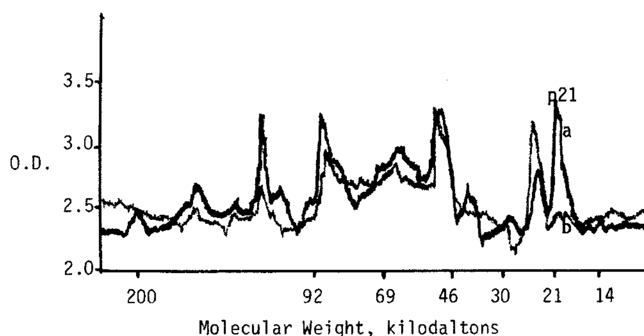

Fig. 5. Densitometer optical density tracings of immunoblots using monoclonal antibody to the peptide sequence of the H-*ras* gene of a serum sample from a worker with heavy carcinogen exposure at: 18 months prior to clinical manifestation of disease *(a)*; 6 weeks after removal of a premalignant lesion *(b)*. Note the peak at 21 kDa in the first sample, which is no longer seen in the second sample

Future research

Further study will obviously be necessary to document the predictive value of the p21 protein in indicating the positive individuals who will actually develop neoplastic disease. A study in a cohort of carcinogen-exposed workers is currently under way for this purpose. In this study, banked sera specimens have been collected annually over the past decade from these workers, a significant proportion of whom now have cancer; the specimens are now being assayed in a blind fashion for the presence of oncogene proteins so as to determine the time course of expression and the ability to predict the subsequent development of disease.

In addition, as knowledge of the biochemistry of the carcinogenic process advances, it is anticipated that new protein biomarkers of cancer, including proteins of other oncogenes and of anti-oncogenes as well as those of other cancer-related genes, can be incorporated into this assay system. For example, although it appears likely that oncogene activation is in most cases related to the initiation stage of carcinogenesis, classic tumor promoters such as phorbol esters have recently been observed to stimulate the expression of the protein of the newly identified phorbin gene, which is also expressed at high levels in carcinogen-induced murine skin tumors and in human colon carcinomas (Johnson et al. 1987a, b). Polyclonal antibodies are available that can detect this protein in cell lysates, and monoclonal antibodies are being developed. These antibodies could be used in the same screening system to assay sera from exposed individuals so as to identify those who are at risk for the development of malignancy due to promotional events.

Finally, in the future, knowledge of the expression of oncogene and other cancer-related proteins in exposed cohorts may serve to direct chemotherapeutic and chemoprophylactic interventions. For example, knowledge as to how the *ras* oncogene becomes activated and as to how structural changes in its protein product produce its transforming effect should allow the design of pharmacologic agents that could interfere with the structure and function of the abnormal protein. Such drugs could be used in the chemotherapy of individuals with malignancies expressing the protein or as chemoprophylaxis in carcinogen-exposed workers who express the protein but do not as yet have clinical disease. We are currently pursuing this line of investigation for the *ras* gene p21 protein. For instance, it is possible to use dialdehyde nucleotide derivatives in vitro that specifically bind to the active site of the p21 protein and thus negate its function (Carucci et al. 1989). Similarly, it is possible to use synthetic peptide analogues of certain critical domains of p21 to negate the protein's function in cells in culture (Lee et al. 1990). Such compounds will serve as prototypes for the development of anti-p21 chemotherapeutic and chemoprophylactic drugs.

In summary, as our understanding of the role of oncogene and other cancer-related proteins advances, it appears increasingly likely that we will be able to exploit this knowledge for preventive purposes in occupational health. In particular, it seems to be very likely that oncogene protein products (p21 and others used in conjunction with markers of biologically effective dose such as PAH-DNA adducts) will be a new and valuable addition to our growing armamentarium of molecular epidemiologic biomarkers of occupational carcinogenesis. Ultimately, it is hoped that the application of such biomarkers will help us to achieve the as yet elusive goal of prevention of all occupational cancer.

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