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Serum Oncogene Proteins in Foundry Workers

PAUL W. BRANDT-RAUF, STEVEN SMITH and FREDERICA P. PERERA
Division of Environmental Sciences, Columbia University School of Public Health, New York, USA

HENRY L. NIMAN
Progenx Inc., San Diego, USA

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

KARI HEMMINKI
Institute of Occupational Health, Helsinki, Finland

REGINA M. SANTELLA
Division of Environmental Sciences, Columbia University School of Public Health, New York, USA

Summary

A new technique for detecting oncogene activation based on immunoblotting for oncogene proteins in serum has been applied to screen a cohort of foundry workers with well-defined workplace exposures to polycyclic aromatic hydrocarbon carcinogens. Three of the 18 individuals screened were found to have abnormal expression of the proteins of the *ras* and *fes* oncogenes. These three individuals were known to have had medium to high workplace exposures to benzo(*a*)pyrene and to have correspondingly high levels of benzo(*a*)pyrene-DNA adducts in their peripheral leukocytes. No individuals among the unexposed controls were found to have abnormal serum oncogene protein expression. These results suggest the feasibility of using serum oncogene proteins along with DNA-carcinogen adducts as potential molecular epidemiological markers in exposed worker populations; further, larger scale studies will be necessary to demonstrate the utility of these markers for identifying individuals at risk for the development of malignant disease due to their occupational exposures.

Introduction

Although still in its infancy, the study of the role of oncogenes in the development of human cancers promises to contribute significantly to our understanding of carcinogenesis and to provide new avenues for early cancer detection and treatment (1). In particular, the detection of activated oncogenes may play a major role in the molecular epidemiological study of cancer evolution in populations environmentally or occupationally exposed to carcinogens (2). For example, it is known that common carcinogens of environmental concern, such as benzo(*a*)pyrene (3), dimethylbenzanthracene (4), *N*-nitroso compounds (5) and ionizing radiation (6), are capable of activating oncogenes. Furthermore, oncogene activation has been found to occur frequently in human tumours of many different types (7) and also in common premalignant lesions (8). This suggests that at least in certain cases oncogene activation may be a sufficiently early step in the carcinogenic process to allow for meaningful intervention once detected, and, thus, the detection of oncogene activation may be a useful marker for identifying individuals at risk for the development of malignancy.

Adapting a urine immunoblotting technique (9), we have recently explored the possibility of using levels of oncogene encoded proteins in serum as markers of carcinogenic response in individuals with cancer or at risk for the development of cancer due to occupational or environmental exposures (10, 11). For example, the sera of a cohort of 18 lung cancer patients (most of whom were cigarette smokers) were screened with a battery of monoclonal antibodies specific for peptide sequences of 9 different oncogenes, and all 18 were positive for at least one oncogene protein in a pattern not normally seen in healthy controls (11). In a study of a cohort of 16 clinically healthy hazardous waste workers with long histories of exposure to multiple carcinogens (including polychlorinated biphenyls, asbestos and cigarette smoke), half of the workers showed some serum oncogene protein abnormality not noted in normal, unexposed controls (10). Furthermore, one of the individuals went on to develop a premalignant colonic lesion, after removal of which, his serum oncogene protein pattern reverted to normal. This case provides support for the concept that serum screening for oncogene proteins may indeed be a useful tool for the early detection and prevention of cancers in individuals with carcinogen exposure (12).

In order to further explore the value of serum oncogene proteins as markers of carcinogenic potential in occupationally exposed populations, this technique has now been applied to a well-defined occupational cohort of iron foundry workers with known quantified exposure to a common occupational carcinogen, benzo(*a*)pyrene. Benzo(*a*)pyrene (BP) and related polycyclic aromatic hydrocarbons (PAHs) have been associated with increased risk of lung cancer in smokers, coke oven workers and foundry workers (13-15), and, as indicated above, such PAHs have been shown to be capable of activating oncogenes both in vitro and in vivo (3, 4). Furthermore, not only are extensive occupational histories available for this cohort, but also their exposure has been well-defined in terms of workplace air levels of BP. In addition, a clear dose-related increase was seen in levels of PAH-DNA adducts in the peripheral leukocytes of these workers (16) which was consistent with measurements of DNA adducts by the ^{32}P-postlabelling method

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(17, 18). This cohort therefore represents a model population for study of oncogene activation related to occupational exposure.

Materials and Methods

The study cohort of workers employed at an iron foundry in Finland has been previously described (16, 18). Briefly, the foundry workers were segregated into groups according to the level of exposure to BP based on extensive industrial hygiene sampling data and job description. This classification was considered representative of exposure during the past 5–10 years, since individuals in this plant tend to remain in the same job and are generally long-term employees. Workers with 8 hour TWA exposures greater than 0·2 $\mu g/m^3$ were classified in the high exposure group and workers with 8 hour TWA exposures between 0·05 and 0·2 $\mu g/m^3$ were classified in the medium exposure group. Clinical information including cigarette consumption was collected on all workers. Unexposed controls for comparison were recruited from patients referred to the Institute of Occupational Health for evaluation of possible occupational disease unrelated to PAH exposure or cancer. The control population had a lower average cigarette consumption than the foundry workers but was similar in terms of age and sex distribution (16).

For the purposes of this study, multiple blood samples were available for three workers in the high exposure group (six blood samples) and five workers in the medium exposure group (twelve blood samples), and single blood samples were available for ten unexposed controls (for a total of 28 blood samples). Two or three blood samples had been collected on the workers at different times. For all workers, samples were available immediately following a month-long vacation and 6 weeks after returning to work. For two of the workers in the medium exposure group, an additional blood sample was collected during the following 12 months. As noted, single blood samples were collected on the unexposed controls. Blood samples (30–50 ml) were collected in heparinized plastic tubes, coded and centrifuged. Buffy coat, red cells and plasma were collected and stored frozen at − 70 °C until time of analysis. PAH-DNA adducts were determined for these same workers as reported previously (16). The values for DNA adducts in a subset of samples assayed for serum oncogene proteins are given in *Table I*; missing values reflect the fact that inadequate amounts of DNA were available for some samples.

These samples have now been analyzed blind for the presence of protein products encoded by nine different oncogenes (*sis*, *fes*, β-TGF, *int*-1, *myb*, *src*, *myc*, *mos*, and *ras*) by the immunoblotting technique as previously described (9, 10). Briefly, 100 μl serum were mixed with 400 μl phosphate-buffered saline (PBS) at pH 7·4, 25 μl 2-mercaptoethanol, and 475 μl sample buffer in deionized water (6·25 per cent sodium dodecyl sulphate, 6·25 per cent glycerol), and placed in a boiling water bath for five minutes. Samples are then loaded on a 5–17 per cent polyacrylamide gel and electrophoretically separated and transferred to nitrocellulose. After blocking with PBS containing 3 per cent bovine serum albumin and 0·1 per cent Triton X-100, the nitrocellulose is incubated overnight at 4 °C with monoclonal antibodies directed against synthetic peptides representing predicted oncogene sequences (ascites fluid diluted 1:2000). After exhaustive washing, the location of the oncogene protein-antibody bands on the nitrocellulose is determined colorimetrically using a secondary anti-mouse IgG antibody and an avidin-biotin-peroxidase complex (Vectastain; Vector Labs, Burlingame, CA). Approximate quantification of positive results is achieved by serial dilution of samples until no difference from normals is detectable; a fivefold increase in protein expression is considered a positive result. The primary antibodies used were directed against the following oncogene protein se-

Table I. Serum oncogene protein expression and PAH-DNA adducts in foundry workers and unexposed controls

Ambient exposure, μg BP/m³	*Patient*	*Smoking, cigarettes/day*	*PAH-DNA adduct levels*, fmol/μg*			*Serum oncogene proteins**					
						fes			*ras*		
			Post-Vacation	*Work 1*	*Work 2*	*Post-Vacation*	*Work 1*	*Work 2*	*Post-Vacation*	*Work 1*	*Work 2*
	1	0	0·11	0·8	NA	−	−	NA	−	−	NA
>0·2	2	20	0·13	2·0	NA	+	+	NA	−	−	NA
(high exposure)	3	0	NA	2·8	NA	−	−	NA	−	−	NA
	4	15	0·13	0·36	NA	−	−	NA	−	−	NA
0·05 – 0·2	5	20–25	NA	0·32	0·42	−	−	−	−	+	−
(medium exposure)	6	15	NA	0·8	0·5	−	−	−	−	−	−
	7	0	0·48	1·28	NA	+	+	NA	−	−	NA
	8	20	ND	0·4	NA	−	−	NA	−	−	NA
	9	15		0·08			−			−	
	10	10		ND			−			−	
	11	0		0·14			−			−	
	12	0		ND			−			−	
<0·05	13	0		ND			−			−	
(unexposed controls)	14	0		0·3			−			−	
	15	0		ND			−			−	
	16	10		ND			−			−	
	17	15		0·1			−			−	
	18	0		0·1			−			−	

NA = not available; ND = non-detectable

* Post-vacation samples were drawn after a one month vacation, work 1 samples were drawn 6 weeks after returning to work; work 2 samples were drawn more than 2 months after returning to work.

quences: *sis* (hybrid 112-09B10, sequence SLGSLTIAE-PAMIAEC), *fes* (hybrid 127-42C11 and 127-50D04, sequence LMEQCWAYEPGQRPSF, and hybrid 121-14C09, sequence IGRGNFGEVFSG(C)); β-TGF (hybrid 100-30C05 and hybrid 100-34E06, sequence ALDT-NYCFSSTEKNC); *int*-1 (hybrid 222-35C08 and hybrid 222-37F04, sequence LHNNEAGRTTVFS(C)); *myb* (hybrid 133-10F06, sequence LGEHHCTPSPPVDHG); *src* (hybrid 203-07D10, sequence (C) GSSKSKPKDPSQRRHS); *myc* (hybrids 155-11C07, 155-08G01 and 155-09F06, sequence CSTSSLYLQDL-SAAASEC); *mos* (hybrid 165-35F02, sequence LGSGGFGSVYKA(C)); *ras* (hybrid 142-24E05, sequence YREQIKRVKDSDDVPMVLVGNKC and hybrid 143-03E04, sequence YTLVREIRQHKL-RKLNPPDESGPGC). In this immunoblotting system, these antibodies have been found to give specific, sensitive and reproducible results. The specificity of the antibodies has been demonstrated by the blocking of the activity by preincubation with the specific peptide for the oncogene and the failure of the blocking of the activity with peptides of other oncogenes (9). The assay system is capable of detecting proteins in the nanogram range and is found to give reproducible results when repeated on the same sample.

Results

Results are presented in *Table I*. No positive bands for the proteins of seven of the oncogenes (*sis*, β-TGF, *int*-1, *myb*, *src*, *myc*, *mos*) were detectable in any of the specimens. Four samples were found to be positive for increased levels of *fes* oncogene-related protein products. These were repeat samples drawn from each of two different workers (patients 2 and 7) post-vacation and after 6 weeks of work and were identical for each patient. One of the workers (patient 2) was in the high exposure category; the other (patient 7) was in the medium exposure category. One sample was found to be positive for *ras* oncogene-related protein products; however, in this case, two other samples from the same worker (patient 5 in the medium exposure group) at different times were negative. Both the post-vacation sample and one of the two post-exposure samples for patient 5 were negative; therefore the significance of the one isolated positive result is less clear. All results for serum oncogene proteins in the unexposed controls were negative.

Discussion

In this study it is notable that the only positive results for elevated serum levels of oncogene-related proteins were obtained in individuals in the medium or high exposure groups. Furthermore, as shown in *Table I* and reported previously (16), these individuals also have high levels of PAH-DNA adducts compared to low exposed or unexposed individuals. Although two of the individuals with positive oncogene proteins were also smokers of approximately one pack of cigarettes per day (patients 2 and 5), it is likely that the major contribution to their body burden of PAHs was due to workplace exposure. For example, exposure at 0·2 $\mu g/m^3$ is approximately equal to the BP dose from smoking 5–7 packs of cigarettes per day.

The positive results for particular oncogene expression in these workers are of interest, since it is known that foundary workers are at increased risk for the development of lung cancer (14). The worker with the isolated positive result for *ras* proteins is notable, since PAHs are known to activate the *ras* gene (3, 4) and *ras* gene activation has been found frequently in human lung cancers, particularly of the non-small-cell variety (7, 19, 20). For example, in a recent study of lung cancer (19), 50 per cent of adenocarcinoma patients (5 of 10) had an activated form of one type of *ras* gene (K-*ras*) and it was felt that this oncogene activation represented a relatively early event in the development of the lung cancers and was related to cigarette smoking. In our studies of serum oncogene protein levels in non-small-cell lung cancer patients, 15 out of 18 patients had increased expression of the *ras* gene products, and 12 of these were known to have been cigarette smokers (12). Increased urinary levels of *ras* oncogene protein products in lung cancer patients have also been demonstrated (9). In our screening of the sera of 16 clinically healthy hazardous waste workers with known carcinogen exposure, one individual had an extremely abnormal increased expression of *ras* gene protein (10). This individual also had the worst history of exposure to carcinogens including asbestos and cigarette smoke. Thus, it is possible that *ras* gene activation plays a significant role in pulmonary carcinogenesis, particularly that caused by exposure to environmental carcinogens such as PAHs. However, in the current study as noted above, the individual (patient 5) with elevated serum *ras* proteins was found to be positive on only one of the three samples. The significance of this isolated finding in terms of this individual's risk for the development of lung or other cancers remains uncertain.

As noted, however, two workers (patients 2 and 7) had consistently elevated serum levels for *fes* oncogene-related protein products. One of these workers was a caster in the Zimmerman process with high exposure to PAHs for the past 15 years, smoked cigarettes and had a father and mother who both died of lung cancer. The other worker had been a core-setter with medium exposure to PAHs for the past 9 years. Both of these individuals also had occupational dermatitis and had long-term treatment with topical steroids. Whether topical exposure to steroids influenced the metabolic activation of PAHs or whether the dermatitis increased dermal absorption of the carcinogens is not clear. Like *ras*, *fes* has been found to be frequently expressed in human lung cancers. In one study, all four lung cancers studied were positive for increased expression of the *fes* gene (7). In our prior study of serum oncogene protein levels in lung cancer patients, 11 of 18 individuals were positive for *fes* products, and 8 of these were known to have been cigarette smokers (11). Increased urinary levels of *fes* oncogene products have also been found in lung cancer patients (9). In addition, in our study of clinically healthy hazardous waste workers, only heavy cigarette smokers (6 of 12) were found to be positive for the *fes* gene products in their serum (10). Furthermore, it is known that during mammalian development, the protein product of the *fes* gene is expressed in a very limited tissue-specific fashion (21); for example, in young chicks 6 to 18 days old, the *fes* gene product is detected at high levels only in three tissues, namely bone marrow, liver and lung (22). Thus, in pulmonary carcinogenesis, the expression of the *fes* gene products may signal a regression to an earlier stage of pulmonary cellular development, and, once again, this

expression may be particularly associated with exposure to the carcinogens of cigarette smoke such as PAHs. Therefore, in this study, it is tempting to speculate that the two workers with known long-term medium-to-high level workplace exposure to PAHs and known high levels of PAH-DNA adducts and with consistently high levels of *fes* oncogene protein products in their serum are the individuals in this cohort most at risk for the development of malignant disease, especially cancer of the lung.

Obviously, these conclusions remain highly speculative, since the total number of available samples and the number of positive results in this cohort are very small and follow-up as to outcome is limited. The interpretation of these results must therefore be approached with considerable caution pending the outcome of more definitive studies. Clearly, long-term follow-up of this cohort is required. More importantly, it will be necessary to perform large-scale longitudinal studies of exposed worker cohorts before the predictive value of serum oncogene proteins or DNA-carcinogen adducts as markers for cancer development can be confirmed. However, the current results suggest the feasibility of using serum oncogene proteins along with DNA-carcinogen adducts as potential molecular epidemiological markers in exposed worker populations.

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REFERENCES

1. Brandt-Rauf PW, Pincus MR. Oncogenes and oncogene proteins. *Occupational Medicine: State of the Art Reviews* 1987; 2: 27.
2. Brandt-Rauf PW. New markers for monitoring occupational cancer: the example of oncogene proteins. *J Occup Med* 1988; 30: 399.
3. Marshall CJ, Vousden KH, Phillips DH. Activation of c-Ha-*ras*-1 proto-oncogene by *in vitro* modification with a chemical carcinogen, benzo(a)pyrene diol-epoxide. *Nature* 1984; 310: 586.
4. Balmain A, Pragnell IB. Mouse skin carcinomas induced *in vivo* by chemical carcinogens have a transforming Harvey-*ras* oncogene. *Nature* 1983; 303: 72.
5. Sukumar S, Pulciani S, Doniger J, DiPaolo JA, Evans CH, Zbar B, Barbacid M. A transforming *ras* gene in tumorigenic guinea pig cell lines initiated by diverse chemical carcinogens. *Science* 1984; 223: 1197.
6. Frazier ME, Lindberg RA, Mueller DM, Gee A, Seed TM. Oncogene involvement in plutonium induced carcinogenesis. *Int J Rad Biol* 1986; 49: 542.
7. Slamon DJ, DeKernion JB, Verma IM, Cline MJ. Expression of cellular oncogenes in human malignancies. *Science* 1984; 224: 256.
8. Spandidos DA, Kerr IB. Elevated expression of the human *ras* oncogene family in premalignant and malignant tumors of the colorectum. *Br J Cancer* 1984; 49: 681.
9. Niman HL, Thompson AMH, Yu A, Markham M, Willems JJ, Herwig KR, Habib NA, Wood CB, Houghten RA, Lerner RA. Anti-peptide antibodies detect oncogene-related proteins in urine. *Proc Natl Acad Sci USA* 1985; 82: 7924.
10. Brandt-Rauf PW, Niman HL. Serum screening for oncogene proteins in workers exposed to PCBs. *Br J Ind Med* 1988; 45: 689.
11. Brandt-Rauf PW, Smith S, Perera FP. Molecular epidemiology and environmental carcinogenesis of the lung. *Current Issues in Respiratory Public Health* 1989; in press.
12. Brandt-Rauf PW. Oncogene proteins as molecular epidemiologic markers of cancer risk in hazardous waste workers. *Occupational Medicine: State of the Art Reviews* 1989; 5: 59–65.
13. Redmond CL, Strobino BR, Cypess RH. Cancer experience among coke by-product workers. *Ann NY Acad Sci* 1976; 271: 102.
14. International Agency for Research on Cancer. Polynuclear aromatic compounds. In: *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Lyon: IARC, 1984; vol. 34 part 3.
15. International Agency for Research on Cancer. Tobacco smoking. In: *Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, Lyon: IARC, 1986; vol. 38.
16. Perera FP, Hemminki K, Young TL, Brenner D, Kelly G, Santella RM. Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundary workers. *Cancer Res* 1988; 48: 2288.
17. Phillips DH, Hemminki K, Alhonen A, Hewar A, Grover PL. Monitoring occupational exposure to carcinogens: detection by ^{32}P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers. *Mutat Res* 1988; 204: 531.
18. Hemminki K, Perera FP, Phillips DH, Randerath K, Reddy MV, Santella RM. Aromatic deoxyribonucleic acid adducts in white blood cells of foundry and coke oven workers. *Scand J Work Environ Health* 1988; 14: 55.
19. Rodenhuis S, van de Wetering ML, Mooi WJ, Evers SG, van Zandwijk N, Bos JL. Mutational activation of the K-*ras* oncogene: a possible pathogenetic factor in adenocarcinoma of the lung. *N Engl J Med* 1987; 317: 929.
20. Kurzrock R, Gallick GE, Gutterman JU. Differential expression of p21 *ras* gene products among histological subtypes of fresh primary human lung tumors. *Cancer Res* 1986; 46: 1530.
21. Pimentel E. *Oncogenes*. Boca Raton: CRC Press, 1986.
22. Mathey-Prevot B, Hanafusa H, Kawai S. A cellular protein is immunologically crossreactive with and functionally homologous to the Fujinami sarcoma virus transforming protein. *Cell* 1982; 28: 897.

Requests for reprints should be addressed to: Dr. P. W. Brandt-Rauf, Director, Occupational Medicine, Division of Environmental Sciences, Columbia University School of Public Health, 60 Haven Avenue, B-1 Level, New York, NY 10032, USA.