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The molecular epidemiology of oncoproteins

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BRANDT-RAUF PW. The molecular epidemiology of oncoproteins. *Scand J Work Environ Health* 1992; 18 Suppl 1:46—9. Oncogene activation, which appears to play a critical role in carcinogenesis, can be conveniently studied in biological samples via immunologic detection of oncoprotein products with monoclonal antibodies. Immunohistochemical and immunocytochemical detection of oncoproteins can be used to differentiate normal cells from cancer cells, including those of cancers of occupational concern such as lung, bladder, and liver. Furthermore, oncoproteins appear to reach the extracellular environment and are detectable with immunoblotting techniques in the urine and serum of cancer patients. Similar techniques applied to the screening of sera of occupational cohorts with carcinogen exposures indicate that oncoproteins can be detected in workers at increased risk of the development of cancer, and thus oncoprotein detection may be a useful molecular epidemiologic biomarker of preclinical response for the surveillance and prevention of occupational cancer.

Key terms: biomarkers, cancer, immunoblotting, immunohistochemistry.

Since oncogene activation may play an important role in certain instances of chemical carcinogenesis, including that due to workplace exposures, the identification of oncogene activation in biological samples offers a potential new method for monitoring the effect of exposure to occupational carcinogens (1). In fact, in certain cases of carcinogenesis, it appears likely that oncogene activation occurs relatively early in the process at the level of premalignant lesions, and thus the identification of oncogene activation in occupationally exposed cohorts could possibly be useful as a marker of preclinical response to carcinogen exposure and could have a significant impact on the prevention of occupational cancer (1). Oncogene activation can be identified at the level of the gene [deoxyribonucleic acid (DNA)], message [ribonucleic acid (RNA)], or protein product (oncoprotein). The identification of oncogene activation via the detection of oncoproteins offers certain advantages over detection at the DNA or RNA level (2). First, the detection of oncogene changes in DNA or RNA does not necessarily guarantee that the change identified has a physiological effect. Oncogenes only exert their transforming effect in cells through the expression of their protein products, and, therefore, it is more likely that detecting the overexpression of an oncoprotein or the expression of a mutated oncoprotein (both of which can cause cell transformation in model systems) will correlate with an effect of clinical significance. Second, the analysis of oncogene changes at the DNA or RNA level requires access to the target cells of interest, which may not be readily obtainable for screening purposes. On the other hand, oncoproteins, as I shall describe, often end up in easily accessible biological fluids, such as serum and urine, and offer a more attractive venue for potential screening tests (1, 2).

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Oncoprotein expression in cancer patients

In order for oncoprotein detection to be useful as a biomarker, it should at a minimum be able to distinguish transformed or cancer cells from nontransformed or normal cells. Such distinction can be accomplished for cells in culture or cancer tissue with immunocytochemical and immunohistochemical staining with monoclonal antibodies that can detect increased amounts of or point-mutated forms of the various oncoproteins (3). The oncoproteins studied best by this technique are those of the *ras* and *myc* oncogenes. The expression of *ras* or *myc* or both oncoproteins has been demonstrated in many different tumor cell lines or cancer tissues (such as breast, colon, stomach, cervix, head and neck and thyroid tissues), including those of occupational concern such as lung, bladder, and liver (4). For example, antibodies to the *ras* p21 oncoprotein have been used to detect elevated levels or aberrant mutant forms in 11 of 23 primary human lung cancers when compared with normal lung tissue (5). The differential expression of p21 in lung cancer may even correlate with histological classification, since, in this study, 9 out of 11 tumors with squamous histology compared with 1 out of 12 nonsquamous carcinomas demonstrated altered p21 expression (5). In another study of human lung cancers, antibodies to the *myc* p62 gene product were used to detect elevated expression in 16 of 37 squamous carcinomas, 4 of 14 adenocarcinomas, and 4 of 21 small cell carcinomas (6). In immunohistochemical studies of human bladder cancers, increased expression of *ras* p21 oncoprotein and

myc p62 oncoprotein appeared to correlate with tumor grade, higher grades having significantly more elevated levels than normal tissue (7, 8). Similarly, in immunohistochemical studies of human hepatocellular carcinomas, an increased expression of p21 and p62 oncoproteins was detectable in a significant proportion of tumors, but not in normal liver tissue (9, 10).

The use of antibodies to detect other oncoproteins in human tumors (including those of occupational concern) has been much less thoroughly studied, but a few examples should be noted. For instance, in comparison with normal tissue, immunohistochemically determined increased expression of the *erbB-1* oncoprotein [epidermal growth factor receptor (EGFr)] has been reported in a high proportion of human squamous-cell lung cancers (11, 12), and an immunohistochemically determined increased expression of the *neu/erbB-2/HER-2* oncoprotein has been reported in a significant proportion of all types of nonsmall cell lung cancers (13). In addition, it should be noted that some of these studies (7, 14) have suggested that altered oncogene expression may occur in a significant proportion of premalignant lesions as well, which, as noted, would have considerable import for potential surveillance application. At any rate, it seems clear from these studies that immunohistochemical techniques for various oncoproteins can be used to distinguish cancers from normal tissue. This type of approach may have useful diagnostic applications for cancer patients, not only for biopsy specimens, but also in the immunocytochemical analysis of more easily obtainable materials such as cytology specimens. Thus, for example, an immunocytochemically determined increased expression of *ras* p21 oncoprotein has been described for the sputum cytology specimens of 61% of squamous-cell lung cancer patients, 32% of adenocarcinoma patients, and 30% of small-cell lung cancer patients (15).

Studies of transformed cells in culture and experimental animal tumors suggest that in certain cases not only are oncoproteins increased inside the cells in these situations, but also that they are shed into the extracellular environment in significant quantities. Thus, with viable transformed cells in culture that are overexpressing the *ras* p21 oncoprotein, elevated amounts of p21 can be detected by Western immunoblotting with monoclonal antibodies in the supernatant (16). Furthermore, if such cells are transplanted into animals and allowed to grow into tumors, elevated levels of p21 can be detected by immunoblotting in the sera of the animals (17). The same situation appears to occur in human cancers such that increased expression of various oncoproteins can be detected by immunoblotting in biological fluids such as urine and serum. For example, 5- to 20-fold increases in the levels of proteins of several oncogenes (*ras, sis, fes*) have been found in the urine of patients with many different types of cancer (including those of occupational concern such as lung and bladder cancer) (18). Elevated levels of oncoproteins have also been detected in the sera of cancer patients. For example, 3 of 13 patients with stomach cancer were reported to have increased levels of p21 in their sera (17). In another study, the sera of 18 patients with histologically documented nonsmall cell cancers of the lung were screened by immunoblotting for the presence of nine different oncoproteins (19). All 18 had altered expression of at least one oncoprotein compared with normal, healthy referents. Fifteen of the 18 were positive for the *ras* p21 oncoprotein, and 10 of the 18 were positive for the *fes* oncoprotein (19).

Furthermore, very preliminary findings suggest that the expression of oncoproteins in the urine and serum of cancer patients may actually precede and predict clinical relapse of disease. For example, in the case of a breast cancer patient in remission, recurrence of an abnormal urinary oncoprotein pattern predated the reappearance of clinical disease by several months (18). In another study, two lung cancer patients who were felt to have had a complete response to therapy were found to have persistently elevated serum levels of p21, a situation suggesting that there must be a residual tumor burden. Within two years, both patients had relapsed and succumbed to their disease (20). These findings, together with the evidence that oncoprotein expression may occur early in the disease process at the level of premalignant lesions, suggest that it may be possible to detect oncoproteins in urine or serum of clinically healthy persons who are at risk for the development of cancer (for instance, due to occupational carcinogen exposures) at a point in the disease process prior to the time of clinical presentation (eg, oncoprotein expression may be able to predict those persons in an exposed worker cohort who will develop neoplastic disease).

Oncoprotein expression in occupationally exposed workers

The immunologic detection of oncoproteins has been applied to the screening of healthy worker cohorts with known occupational exposure to carcinogens. For example, *ras* p21 oncoprotein has been immunocytochemically detected in the cells of an otherwise normal sputum cytology specimen of a smoking asbestos worker, an individual who would clearly be considered to be at increased risk for the development of lung cancer due to those exposures (16). More importantly, perhaps, immunoblotting has been used to detect oncoproteins in the serum of exposed worker cohorts. For example, the sera of 18 Finnish foundry workers with known high ambient workplace exposures to polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene (BaP), known high levels of PAH adducts to DNA in their peripheral lymphocytes, and a known high risk for the development of lung cancer were screened for altered expression of nine different oncoproteins (21). One person in the high-exposure group

(8-h time-weighted average >0.2 μg BaP · m^{-3}) was positive for the *fes* oncoprotein, and two persons in the medium-exposure group (8-h time-weighted average 0.05—0.2 μg BaP · m^{-3}) were positive for the *fes* or *ras* oncoprotein, and no positives for serum oncoproteins were identified among the unexposed referents. In addition, the persons who were oncoprotein positive had a greater than twofold higher average level for PAH adducts to DNA than the oncoprotein negative persons (21). In another study of firefighters, a group suspected to be at increased risk for several different types of cancer, 14 of 33 of the firefighters were found to have elevated serum levels of a growth factor type of oncoprotein (B-transforming growth factor) compared with none of 16 positives among the unexposed referents (Ford J, Smith S, Luo JC, et al, submitted for publication). A third study examined serum oncoproteins among hazardous waste workers with long histories of known multiple carcinogen exposures. Eight of 16 workers in the exposed cohort were found to be positive for various oncoproteins (*ras, fes, sis*), whereas only 2 of 17 referents matched for age, gender, and race were positive. Furthermore, both of the positive referents were found to be heavy cigarette smokers, and all of the nonsmoking referents were negative (22—24).

The follow-up of worker cohorts such as these should help to establish whether or not persons who are exposed to carcinogens and express oncoproteins are at increased risk for the development of neoplastic disease. For example, in the aforementioned study of hazardous waste workers, the individual with the worst history of occupational carcinogen exposure and the highest level of expression of the ras p21 oncoprotein remained clinically healthy for 18 months, but he then developed rectal bleeding and was found to have a premalignant colonic polyp. It was postulated that this person's prior heavy asbestos and possibly other carcinogen exposures may have contributed to his colonic neoplasm. At any rate, upon removal of the polyp, the worker's serum p21 pattern reverted to normal (25). Further support for a possible predictive value of oncoprotein expression in cancer development is provided by the preliminary results of screening of the banked serum specimens of a cohort of 46 asbestos- and silica-exposed workers. Fourteen of these persons have subsequently developed cancer (nine of the respiratory tract, including one mesothelioma). Of the nine respiratory tract cancer cases, seven were found to be positive for serum oncoproteins, and on the average their serum specimens were positive 14 months prior to the time of the clinical diagnosis. Furthermore, the two negative cases that subsequently developed lung cancer had their last blood samples drawn more than two years prior to the time of diagnosis and had no available samples from the time of diagnosis (Brandt-Rauf PW, Hemminki K, Smith SJ, et al, work in progress). These preliminary results tend to suggest that serum oncoprotein expression may be a useful early marker of preclinical response in persons with carcinogen exposure.

Concluding remarks

Due to the apparent critical role of oncogene expression in human carcinogenesis, including that due to chemical exposures, the use of oncoprotein biomarkers as potential molecular epidemiologic tools in occupational cancer surveillance deserves further attention. The detection of oncoproteins may be a useful marker of preclinical response to carcinogen exposures, which, together with other markers of exposure, could contribute significantly to the prevention of occupational cancer.

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