Scand J Work Environ Health 1992;18 Suppl 1:27—30

Oncogenes and oncoproteins in occupational carcinogenesis

by Paul W Brandt-Rauf, MD[1]

BRANDT-RAUF PW. Oncogenes and oncoproteins in occupational carcinogenesis. *Scand J Work Environ Health* 1992;18 Suppl 1:27—30. It seems increasingly likely that an important mechanism of action of certain workplace carcinogens in contributing to occupational carcinogenesis may be via the activation of cellular oncogenes, which then cause an expression of mutated forms or increased amounts of their oncoprotein products. Two prototypical models of this mechanism may be the *ras* oncogene and its p21 protein and the *neu* oncogene and its p185 protein. Both are known to be activated by exposure to common occupational carcinogens, and both are known to occur frequently in human tumors, including those of occupational concern such as lung cancer. Knowledge of their mechanisms of action may lead to new opportunities for preventing occupational cancer.

Key terms: ras, p21, *neu*/c-*erbB*-2/*HER*-2, p185.

In order for a potentially carcinogenic agent in the workplace to cause cancer in a worker, it is known that a number of steps must occur. These include transport of the agent to the worker, absorption of the agent, transport to the target cell, in many cases metabolism of the agent to a reactive species, and interaction with critical molecules in the target cell. In the case of initiating carcinogens, the best studied group of carcinogens, this interaction has been demonstrated to occur at the level of the cell deoxyribonucleic acid (DNA) and to result in some irreversible alteration in the cellular genome, for example, the production of chromosome breaks and rearrangements or the introduction of point mutations (1). How these genomic alterations (together with the less well-defined epigenetic processes that may be involved in the multiple stages of promotion and progression) eventuate in the expression of the malignant phenotype in cells and the production of a cancerous growth remains less certain. However, it seems clear that it is not random alteration of the cellular genome that is responsible for this process, but rather that it is alterations of particular small subsets of genes that make the most important contributions to oncogenesis. These subsets of genes may be further differentiated into those which exert a stimulatory influence on cell growth and division, and thus whose activation can contribute to carcinogenesis (the oncogenes), and those which exert an inhibitory influence on cell growth and division, and thus whose inactivation can contribute to carcinogenesis (the antioncogenes) (1). Although several antioncogenes have been clearly identified (eg, the retinoblastoma gene and the p53 gene) and others are suspected to exist from chromosomal deletion studies of human tumors (eg, defects at 1p in neuroblastomas, 3p in lung and renal cancers, 5q in colon cancers, 11p in Wilm's tumors and bladder cancers, and 22q in acoustic neuromas), the possible role of environmental or occupational carcinogens in the inactivation of these genes has not been investigated, and they will not be considered further in this review (2, 3). On the other hand, it appears increasingly likely that environmental and occupational carcinogens can play a role in the activation of certain oncogenes, and thus oncogenes and their protein products are likely to play an important role in occupational carcinogenesis (4, 5).

Oncogenes and oncoproteins

The oncogene theory of carcinogenesis is based on the premise that there are preexisting, normally innocuous genes in the human genome (protooncogenes) which become inappropriately activated during the process of carcinogenesis, which changes them into genes capable of causing the active transformation of cells (oncogenes). Most such oncogenes were initially discovered as the transformation-causing agents in acute transforming retroviruses. However, it has been subsequently shown that these oncogenes were not native to the viruses but had been appropriated by the virus from mammalian genomes during the course of evolution. These genes presumably exist in the human genome because they play vital roles in normal cell growth, division, and differentiation. However, when they are inappropriately activated, they can cause the neoplastic transformation of cells. To date, several dozen different protooncogenes have been identified (6).

As with all genes, oncogenes produce their effects in cells through the action of their protein products. The oncoproteins can be classified into various types based upon their presumed function in cells as follows (7): (i) growth factors, which are usually proteins secreted by one cell that can then stimulate growth in

[1] Columbia University, 60 Haven Avenue, B-1, New York, New York, United States.

Reprint requests to: Dr PW Brandt-Rauf, Director of Occupational Medicine, Columbia University, 60 Haven Avenue, B-1, New York, NY 10032, USA.

other cells upon interaction with an appropriate receptor molecule [examples include the transforming growth factors (TGF) and platelet-derived growth factor (PDGF;*sis*)]; (ii) membrane receptors, which usually contain an extracellular receptor domain for interaction with a growth factor, a transmembrane domain, and an intracellular domain usually with kinase activity for the phosphorylation of intracellular proteins [examples include the epidermal growth factor receptor (EGFr;*erbB*-1) and the *neu* protein (*erbB*-2;*HER*-2)]; (iii) signal transducing G proteins, which are membrane-associated and presumably participate in signal transduction via their guanosine triphosphatase activity [examples include the *ras* gene proteins (p21)]; (iv) protein kinases, which may or may not be membrane-associated but which presumably participate in signal transduction via the phosphorylation of other intracellular proteins [examples include the *src, fes,* and *mos* gene proteins]; (v) nuclear proteins, which presumably bind to DNA and influence the expression of other genes [examples include the *myc, myb, fos,* and *jun* proteins]. As can be seen from this list, the protein products of most oncogenes appear to play some role in growth signaling (from extracellular growth factors, through the cell membrane, into the cytoplasm, and eventually to the nucleus), consistent with the growing evidence that carcinogenesis represents a progressive disorder of signal transduction in cells (8).

It appears that protooncogenes can be activated to induce cell transformation through their protein products by the following two general mechanisms: overexpression of the normal protein product or expression of a mutated protein product. Overexpression can be achieved by several mechanisms (eg, amplification of the number of copies of the gene present in cells (if each copy is being transcribed and translated, the result will be an increase in the protein product in the cell) or the introduction into the cell of a gene deregulatory sequence (such as a viral long terminal repeat) which could cause uncontrolled and hence increased expression. Alternately, gene overexpression can occur in some instances where chromosomal breaks and rearrangements (as might be inducible by environmental clastogens) result in protooncogenes that thereby lose their normal expression controls. For example, in certain instances, this may be true for the *myc* oncogene. Finally, certain protooncogenes that have undergone point mutations (once again, as could be produced by various environmental mutagens) can cause cell transformation by expressing normal levels of their mutated protein products. This is true, for example, of the *ras* and *neu* oncogenes, both of which can be activated by overexpression of the normal protein products as well, and, thus, they represent interesting paradigms of oncogene-induced carcinogenesis (6).

The *ras* and *neu* oncogenes and their protein products are also of particular interest from the point of view of occupational carcinogenesis since they can be activated by exposure to well-known occupational carcinogens and they are frequently found activated in human malignancies, including those of particular occupational concern such as lung tumors (6). Thus further examination of the possible action of these oncogenes in cells seems warranted.

Ras oncogene and p21 protein

The *ras* oncogene was first identified in acute transforming retroviruses that induced rat sarcomas, hence the name. The gene was also identified in a human bladder carcinoma cell line and shown to be identical to a normal human protooncogene except for a single base change in the 12th codon of the first exon. This point mutation resulted in a single amino-acid substitution in the 12th amino acid of the encoded oncoprotein (a protein of 189 amino acids of molecular weight 21 KD, hence designated p21). It was subsequently shown that overexpression of nonmutated p21, as well as expression of *ras* proteins with other amino-acid substitutions (including most other substitutions at position 12 and substitutions at positions 13, 59, 61, and 63), resulted in cell transformation (6, 9).

As noted, it has been shown in vitro and in animals that the *ras* gene can be activated by exposure to carcinogens of environmental and occupational concern, such as polycyclic aromatic hydrocarbons (PAH) (including benzo[a]pyrene and dimethylbenzanthracene), N-nitroso compounds, and ionizing radiation (9, 10). In addition, the *ras* gene has been found to be frequently activated in human tumors, including those of occupational concern such as lung cancer. For example, a specific form of activated *ras* oncogene was found in 50% (5 of 10) of patients with adenocarcinomas of the lung (11). All five patients with *ras*-positive cancers had been heavy cigarette smokers, whereas two of the patients with *ras*-negative cancers had never smoked and a third had stopped smoking 13 years prior to clinical presentation, thus a possible link in humans between exposure to carcinogens in cigarette smoke (such as PAH) and *ras* oncogene activation in lung cancer being supported (11). Therefore, it would be anticipated that the *ras* gene and its p21 protein would play an important role in certain cases of occupational carcinogenesis.

From experiments in cell culture, it is clear that it is the p21 protein product that is responsible for the transforming activity of activated *ras* genes in cells. For example, in microinjection experiments, when activated p21 is introduced into nontransformed cells in cultures that contain the normal *ras* protooncogene, transient cell transformation is produced, the cells gradually returning to phenotypic normality as the protein is degraded intracellularly (12). Alternately, when monoclonal antibody directed against activated p21 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 (13). Unfortunately, the exact mechanism of action of p21 in cells remains to be elucidated. Activating point mutations in the p21 protein appears to cause conformational changes in the protein structure, and this phenomenon may explain its transforming activity (14). Protein conformation of p21 may also explain the activating effect of *ras* overexpression because of the fact that, even for the normal protooncogene-encoded proteins, a small proportion of molecules exists in the same conformation as that 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 physiological effect, but, with overexpression, a sufficient number of protein molecules in this minority conformation is generated to result in cell transformation (15). These conformational changes may relate to differences in GTPase activity between normal and activated p21. All p21 proteins bind guanosine 5'-triphosphate, but the normal protein contains GTPase activity that is about an order of magnitude greater than that for the activated protein due to the differential effect of GTPase-activating protein (GAP). This GAP protein is thus clearly an upstream regulator of p21 signal-transducing activity, but its potential role in downstream signal transduction is unclear. It is clear that p21 interacts with several other intracellular proteins, and these may be important in downstream signal transduction. It has been proposed that this signal transduction pathway involves phospholipase C-phosphatidylinositol mechanisms, but this proposal remains to be definitively established (9).

Neu oncogene and p185 protein

The *neu* oncogene was first identified in a model animal system dependent on chemical induction of tumors by alkylating agents such as *N*-nitroso compounds. For example, exposure of perinatal BDIX rats to a single dose of ethylnitrosourea led to a high incidence of neuroectodermal tumors in the animals (16). DNA isolated from four independently derived tumor cell lines of this type was shown to contain activated oncogenes as detected in an NIH 3T3 focus-forming assay, and one of these genes was designated *neu* (17). The activated form of the gene was found to contain a single point mutation which resulted in a single amino-acid substitution at position 664 in the encoded oncoprotein (a protein of molecular weight 185 KD, hence designated p185) (18). It was subsequently shown that other selected amino-acid substitutions at position 664, as well as overexpression of the nonmutated p185, resulted in cell transformation (19, 20). Homologous human versions of the *neu* gene have also been identified and termed variously c-*erbB*-2 or *HER*-2 (6).

Like *ras*, not only is the *neu* oncogene known to be activatable by exposure to carcinogens of environmental and occupational concern (ie, *N*-nitroso compounds), but also the gene is frequently found in human tumors of several types including those of occupational concern such as lung cancers. For example, *neu*/c-*erbB*-2/*HER*-2 has been found in 8 of 22 human nonsmall cell carcinoma cell lines and in human tumor tissue from 4 of 12 adenocarcinomas of the lung and 3 of 5 squamous cell carcinomas of the lung (21). Thus, as in the case of *ras*, it would be anticipated that the *neu* gene and its p185 protein would play an important role in certain cases of occupational carcinogenesis.

The *neu*-encoded p185 protein has extensive homology with the epidermal growth factor receptor (EGFr) (6). Like EGFr, p185 has a large cysteine-rich extracellular domain of 650 amino acids, a single transmembrane domain, and an intracellular domain of 580 amino acids with tyrosine kinase activity (6). On the basis of this homology, it has been postulated that p185 is the receptor for an as yet unidentified growth factor and, furthermore, that the binding of this factor promotes receptor dimerization which stimulates the cytoplasmic tyrosine kinase activity of p185, which causes signal transduction into the cell (19). A potential candidate for this growth factor has recently been identified (22). As noted, the transforming p185 produced in animals by exposure to *N*-nitroso compounds has a single amino-acid substitution at position 664 in the transmembrane domain. The location of this activating mutation in the transmembrane domain indicates an important role for this domain in regulating the activity of the oncoprotein and its normal counterpart, perhaps, as has already been noted, by promoting dimerization within the cell membrane and stimulating tyrosine kinase activity. In fact, it has been shown that the presence of activating substitutions at position 664 does lead to aggregation of *neu* proteins in the membrane (23). Therefore, it is possible that activating mutations in p185 or overexpression of normal p185 may lead to signal transduction from membrane aggregation in the absence of a growth factor-receptor interaction, thus the transforming effects observed being explained. As in the case of p21, it appears likely that this process is mediated by conformational changes produced in the transmembrane domain of p185 by the mutations or, also as in the case of p21, during overexpression of *neu* by the production of normal p185 in an amount sufficient enough to have a similar effect in a minority transforming conformation (24, 25). The subsequent pathway of signal transduction from p185 into the cell remains to be elucidated.

Concluding remarks

It seems increasingly likely that certain oncogenes (especially, as has already been noted, *ras* and *neu*), via expression of mutated forms or increased amounts of their protein products, will be found to play critical

roles in some cases of occupational carcinogenesis. A better understanding of the mechanisms of action of these oncogenes and their oncoproteins at a molecular level could thus have a profound influence on the study and prevention of occupational cancer. For example, oncogenes and oncoproteins may serve as useful biomarkers in the molecular epidemiologic surveillance of workers exposed to occupational carcinogens (5). In addition, knowledge of oncogene activation and oncoprotein structure and function may lead to new approaches to cancer treatment and cancer chemoprophylaxis based on the development of oncogene- or oncoprotein-specific interventions (26, 27; Chung DL, et al, submitted for publication). Ultimately, it is hoped that such approaches will contribute to a significant reduction in many types of cancer, including those due to workplace exposures.

Acknowledgments

This work was supported in part by Public Health Service Grants OH00076 and CA42500 and grants from International Business Machines, EI Dupont de Nemours and Co, the Samuel and May Rudin Foundation, and the Lucille P Markey Trust.

References

1. Hermo H, Brandt-Rauf PW. Chemically induced oncogenesis. Semin Occup Med 1987;2:235—43.
2. Weinberg RA. Oncogenes, antioncogenes and the molecular bases of multistep carcinogensis. Cancer Res 1989;49:3713—21.
3. Levine AJ, Momand J. Tumor suppressor genes: the p53 and retinoblastoma sensitivity genes and gene products. Biochim Biophys Acta 1990;1032:119—36.
4. Brandt-Rauf PW, Pincus MR. Oncogenes and oncogene proteins. In: Brandt-Rauf PW, ed. Occupational cancer and carcinogenesis. Philadelphia, PA: Hanley & Belfus Inc, 1987:27—38. (Occupational medicine: state of the art reviews; vol 2.)
5. Brandt-Rauf PW. Oncogene proteins as biomarkers in the molecular epidemiology of occupational carcinogenesis: the example of the *ras* oncogene encoded p21 protein. Int Arch Occup Environ Health. In press.
6. Pimentel E. Oncogenes. Boca Raton, FL: CRC Press, 1989.
7. Hurst RE, Jones PL, Rao JY, Hemstreet GP. Molecular and cellular biological approaches and techniques in the detection of bladder cancer and enhanced risk for bladder cancer in high-risk groups. J Occup Med 1990;32: 854—62.
8. Weinstein IB. Growth factors, oncogenes, and multistage carcinogenesis. J Cell Biochem 1987;33:213—24.
9. Barbacid M. *Ras* genes. Annu Rev Biochem 987;56: 779—827.
10. Balmain A, Brown K. Oncogene activation in chemical carcinogenesis. Adv Cancer Res 1988;51:147—82.
11. Rodenhuis S, van de Wetering ML, Mooi WJ, Evers SG, van Zandwijk W, 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—35.
12. Stacy DW, Kung HF. Transformation of NIH 3T3 cells by microinjection of Ha-*ras* p21 protein. Nature 1984; 310:508—11.
13. Feramisco JR, Clark R. Wong G. Transient reversion of *ras* oncogene-induced cell transformation by antibodies specific for amino acid 12 of *ras* protein. Nature 1985;314:639—43.
14. Pincus MR, Brandt-Rauf PW. Structural effects of substitutions on the p21 proteins. Proc Natl Acad Sci USA 1985;82:3596—600.
15. Pincus MR, Brandt-Rauf PW. Protein structure and cancer. Cancer Invest 1986;4:185—95.
16. Rajewski MF, Augenlicht LH, Biessman H, Goth R, Huelser DF, Laerum OD, et al. Nervous-system specific carcinogenesis by ethylnitrosourea in the rat: molecular and cellular aspects. In: Haitt H, Watson J, Winsten J, ed. Origins of human cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977:709—26.
17. Shih C, Padhy LC, Muray M, Weinberg RA. Transforming genes of carcinomas and neuroblastomas introduced into mouse fibroblasts. Nature 1981;290: 261—4.
18. Padhy LC, Shih C, Cowing D, Finkelstein R, Weinberg RA. Identification of a phosphoprotein specifically induced by the transforming DNA of rat neuroblastomas. Cell 1982;28:865—71.
19. Bargmann CI, Weinberg RA. Oncogenic activation of the *neu*-encoded receptor protein by point mutation and deletion. EMBO J 1988;7:2043—52.
20. Hudziak RM, Schlessinger J, Ullrich A. Increased expression of the putative growth factor receptor p185 *HER2* causes transformation and tumorigenesis of NIH/3T3 cells. Proc Natl Acad Sci USA 1987;84:7159—63.
21. Weiner DB, Nordberg J, Robinson R, Nowell PC, Gazdar A, Greene MI, et al. Expression of the *neu* gene-encoded protein (p185*neu*) in human non-small cell carcinomas of the lung. Cancer Res 1990;50:421—5.
22. Lupu R, Colomer R, Zugmaier G, Sarup J, Shepard M, Slamon D, et al. Direct interaction of a ligand for the *erbB2* oncogene product with the EGF receptor and p185 *erbB2*. Science 1990;249:1552—5.
23. Weiner DB, Liu J, Cohen JA, Williams WV, Greene MI. A point mutation in the *neu* oncogene mimics ligand induction of receptor aggregation. Nature 1989;339: 230—1.
24. Brandt-Rauf PW, Pincus MR, Chen JM. Conformational changes induced by the tranforming amino acid substitutions in the transmembrane domain of the *neu* oncogene encoded p185 protein. J Protein Chem 1989; 8:749—56.
25. Brandt-Rauf PW, Rackovsky S, Pincus MR. Correlation of the structure of the transmembrane domain of the *neu* oncogene-encoded p185 protein with its function. Proc Natl Acad Sci USA 1990;87:8660—4.
26. Carucci JE, Chung DL, Carty RP, Murphy RB, Chen J, Kung HF, et al. Blocking of the nucleotide exchange of the p21 protein with an active site affinity label. Med Sci Res 1989;17:559—60.
27. Lee G, Ronai ZA, Pincus MR, Brandt-Rauf PW, Murphy RB, Delohery TM, et al. Inhibition of *ras* oncogene-encoded p21 protein-induced pinocytotic activity by a synthetic peptide corresponding to an effector domain of the protein. Med Sci Res 1990;18:771—2.