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Cellular and Molecular Mechanisms of Multistep Carcinogenesis: Relevance to Carcinogen Risk Assessment

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Carcinogenesis is a multistep process involving alterations in at least two distinct classes of genes. Protooncogenes are activated qualitatively or quantitatively in certain tumors, and they appear to act as positive proliferative signals for neoplastic growth. In contrast, tumor suppressor genes are normal genes that must be inactivated or lost for tumor development. When active, tumor suppressor genes control neoplastic growth in a negative manner. Chemicals may influence the carcinogenic process by mutational activation of protooncogenes and/or inactivation of tumor suppressor genes. The types of genetic alterations involved in these mutational events are diverse, and their dose-response curves may be varied. In addition, chemical carcinogens may act on nonmutational processes such as the clonal expansion of premalignant cells. The carcinogenic risk of a specific chemical is a composite of its effects on multiple genetic and epigenetic processes.

Carcinogenesis is a Multistep Process

It is generally accepted that chemical carcinogenesis is a multistep process (Fig. 1). Several lines of evidence supporting this conclusion are listed in Table 1 and discussed in detail elsewhere (*1*). One of the underlying premises of most multistep models of carcinogenesis is that genetic and/or epigenetic alterations of multiple, independent genes are involved. While the process of chemical carcinogenesis can often be separated operationally into at least three stages, i.e., initiation, promotion, and progression (*2*), it is not clear how many genetic changes are involved in these operationally defined stages.

Initiation involves the induction of an irreversibly altered cell and is frequently equated with a mutational event. This conclusion is supported by the recent findings of mutational activation of *ras* protooncogenes in rat mammary carcinomas, mouse skin papillomas, and mouse hepatomas (*3–7*). The mechanisms of initiation may vary, however, in different tissues or with different initiators in the same tissue (*4,5*). Promotion is the experimentally defined process by which the initiated cell clonally expands into a visible tumor, often a benign lesion such as a papilloma. This process undoubtedly involves epigenetic factors that selectively influence the proliferation of the initiated cell. Whether genetic mechanisms are also involved in this process is unclear.

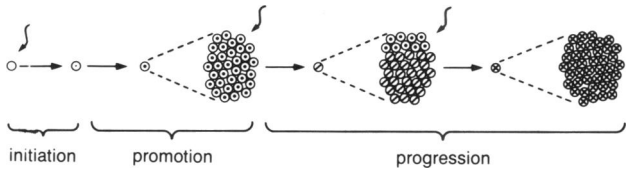

FIGURE 1. In this model of neoplastic development it is assumed that the heritable alterations of different genes occur as the consequence of chemically induced or spontaneous events.

For risk assessment it is important to understand how a chemical affects all stages in the carcinogenic process. For example, urethane (ethyl carbamate) is not a complete carcinogen in mouse skin, although it is an effective initiator (*8*). This is presumably due to its inability to elicit epidermal hyperplasia and to act a tumor promoter (*8*). On the other hand, urethane is an effective hepatic carcinogen in weanling mice, presumably because cell division in the liver during development acts as an effective promoting stimulus (*9*). Phorbol esters are active tumor promoters (*10*), but weak complete carcinogens (*11*). This is probably due to their inability to act as initiators (*10*). Thus, the lack of effective initiating or promoting activities can limit the carcinogenicity of a chemical in certain contexts.

The end product of tumor promotion is generally a benign lesion or preneoplastic foci of cells. These cells must undergo one or more additional heritable changes during the progression to a malignant neoplasm. The progression of benign tumors to malignant cancers is a phase in carcinogenesis clearly distinct from promotion.

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Table 1. Evidence for multistage models of carcinogenesis.

Histopathological observations of tumors reveal multiple stages of tumor progression such as dysplasia and carcinoma *in situ*
Two-stage model of chemical carcinogenesis in mouse skin shows that different chemicals affect qualitatively different stages in the carcinogenic process
Individuals with genetic traits manifested by an early occurrence of cancer (e.g., familial retinoblastomas, adenomatosis of the colon and rectum) suggest that one step in the carcinogenic process can be a germline mutation, but additional somatic events are required for neoplastic development
Mathematical models based on age-specific tumor incidence curves are consistent with four to seven independent hits required for tumors
Cell culture studies with chemical carcinogens reveal that different phenotypic properties of a tumor cell are acquired by a progressive process
Cell culture studies with viral and tumor-derived oncogenes show that neoplastic conversion of normal cells generally requires multiple cooperating oncogenes. In constrast, certain preneoplastic (immortal) cells are neoplastically transformed by a single oncogene
Transgenic mice that carry activated protooncogenes in their germline develop focal tumors that are apparently monoclonal in origin, suggesting that additional somatic events are required for full malignant progression

This conclusion is supported by a number of observations. Malignant tumors are distinct from benign tumors or other preneoplastic lesions in terms of their histopathological characteristics of cellular morphology, invasiveness, growth, and differentiation. The stages of promotion and progression can also be distinguished on the basis of differential responses to certain chemical treatments. In initiation-promotion experiments on mouse skin, the incidence of carcinomas is not necessarily proportional to the number of papillomas (*12–17*). Telocidin, an indole alkaloid, induces more carcinomas, but fewer papillomas, than the phorbol ester promoter 12-*O*-tetradecanyolphorbol-13-acetate (TPA) on 7,12-dimethylbenz[a]anthracene (DMBA)-initiated mouse skin (*12*). Mezerein is a weak promoter of epidermal papillomas in SENCAR mice, but it yields a similar number of carcinomas as the potent promoter TPA (*13*). Likewise, the free-radical-generating chemical benzoyl peroxide is only moderately active as a tumor promoter but is far more active than TPA in inducing malignant tumors (*14*). Finally, the anti-inflammatory steroid fluocinolone acetonide inhibits DMBA-initiated, 7-bromomethylbenz[a]anthracene-promoted papillomas without affecting the carcinoma incidence in treated mice (*17*). These studies clearly indicate that the induction of carcinomas is only in part determined by the number and nature of the benign papillomas. Tumor promoters, although effective in producing multiple benign tumors or preneoplastic foci, are not particularly effective in influencing the progression of these lesions to malignant neoplasms in many experimental models (*18,19*). For example, in the mouse skin model, phorbol ester tumor promoters influence progression by increasing the number of precursor lesions (i.e., papillomas), but do not directly induce the transition of papillomas to carcinomas (*18,20*). Treatment of benign tumors with alkylating and other mutagenic agents increases the frequency and rate of malignant conversion (*20,21*). The promoter TPA is ineffective in enhancing malignant progression, but other promoters (e.g., benzoyl peroxide and telocidin) may effect both promotion and progression (*12,14*). These observations are important in understanding why treatment with complete carcinogens is much more effective than initiation-promotion protocols for the induction of malignant tumors (*22*).

Thus, the evolution of malignant tumors from benign lesions involves the acquisition of one or more qualitative changes in the precursor cells. In fact, progression probably involves multiple, heritable changes. In mouse skin, papillomas display no histopathological evidence of dysplasia after 10 weeks of tumor promotion with phorbol esters (*23,24*); however, at later times (20–40 weeks of promotion), the papillomas show evidence of moderate to severe dysplasia and concomitantly, aneuploid tumor cells are detectable. These phenotypic changes are also observed in the carcinomas that arise from these papillomas (*23*). In chemically induced rat hepatocarcinogenesis, multiple events are postulated to be involved in the progression phase (*25,26*). In other tissues, morphological evidence for multiple steps in the progression from dysplastic lesions to carcinomas *in situ* and ultimately to malignant carcinomas is well established (*27*).

From epidemiological studies, some human carcinogens have been shown to affect predominately late stages in the carcinogenic process (*28*). This does not necessarily imply that such chemicals operate similarly to tumor promoters in two-stage experimental models. The chemical may affect events in the progression phase of carcinogenesis, which, as described above, are not affected by classical promoters such as the phorbol esters. Arsenic is an example of a chemical that may act primarily as a progressor, i.e., a chemical that affects the progression stage. Arsenic is a well-established carcinogen in humans (*29,30*), but there is little evidence for its carcinogenicity in animals (*31–33*). It is inactive as an initiator or tumor promoter in a two-stage model of epidermal carcinogenesis in mice (*34,35*). Brown and Chu (*36*) have proposed that arsenic exposure affects a late stage in the carcinogenic process based on exposure effects in humans. These authors have further postulated that the human data are inconsistent with the hypothesis that arsenic acts during the promotion phase of the carcinogenic process. This conclusion is based on the epidemiological data that do not show reversibility of the excess lung cancer mortality after exposure ceases. Reversibility is one of the hallmarks of tumor promotion (*10*). Based on these observations, we have proposed that arsenic acts specifically in the progression phase of carcinogenesis (*19*). This hypothesis is supported by our recent observation that arsenic is an ef-

fective inducer of gene amplification (*37*) and would explain why arsenic is ineffective as a complete carcinogen, initiator, or tumor promoter. Oncogene amplification has been shown in some tumors to correlate with the degree of neoplastic progression (*38–40*), and arsenic-induced oncogene amplification may explain the observed increase of tumor incidence at a late stage in human carcinogenesis. These findings emphasize the importance of all the steps in the multistep process of carcinogenesis. Risk assessment based only on the principles of initiation and promotion will not accurately predict the hazards of chemical carcinogens.

Carcinogenesis Involves Changes in Two Classes of Genes: Protooncogenes and Tumor Suppressor Genes

Another approach to understanding the influence of chemicals on carcinogenesis is to define the basis for the heritable changes in cancer cells and to elucidate the mechanisms by which chemicals affect these alterations. New understandings of the number and types of genetic changes involved in the conversion of a normal cell into a malignant cell are beginning to emerge. At least two classes of genes have been shown to be involved in carcinogenesis: protooncogenes and tumor suppressor genes (Table 2). Protooncogenes are a family of cellular genes with at least 30 members that appear to be involved in normal cellular growth and development; activation or inappropriate expression of these genes results in proliferative signals involved in neoplastic growth (*41,42*). On the other hand, tumor suppressor genes are less well defined, but the function of these genes may also be to control cell division and possibly differentiation (*43–48*). For a tumor cell to emerge, these genes must be inactivated or lost (*49,50*). The number of tumor suppressor genes is unknown, but multiple genes are likely to exist, possibly with limited tissue specificity (*44,48*).

Table 2. Two classes of genes involved in carcinogenesis.

Protooncogenes	Tumor suppressor genes
Involved in cellular growth and differentiation	Function unknown but possibly involved in cellular growth and differentiation (negative regulators of cell growth?)
Family of genes exists	Family of genes exists
Must be activated (quantitatively or qualitatively) in cancers	Must be inactivated or lost in cancers
Mutational activation by point mutation, chromosome translocation, or gene amplification	Mutational inactivation by chromosome loss, chromosome deletion, point mutation, somatic recombination or gene conversion
Little evidence for involvement in hereditary cancers	Clear evidence for involvement in hereditary and nonhereditary cancers
Limited tissue specificity for members of the *ras* gene family	Considerable tissue specificity

Table 3. Molecular and cytogenetic examples of mutational changes in tumors.

Type of genetic change	Examples
Gene mutation	Point mutation (G→T) in codon 12 (gly→val) of the c-Ha-*ras* gene in EJ/T-24 human bladder carcinoma Point mutation (G→A) in codon 12 (gly→glu) of the c-Ha-*ras* gene in MNU-induced mammary carcinomas Point mutation (A→T) in codon 664 (val→glu) of the *neu* gene in ethylnitrosourea-induced neuroblastomas
Chromosome rearrangement	Philadelphia translocation [t(9;22) (q34; q11)] in human chronic myelogenous leukemia (CML) in which the c-*abl* protooncogene is fused to the *bcr* gene by a translocation to chromosome 22q Burkitt's lymphoma in which the c-*myc* protooncogene is frequently translocated to chromosome 14q, in the region coding for the immunoglobulin heavy chain
Gene amplification	N-*myc* gene in neuroblastomas c-*myc* gene in human lung carcinomas *neu* gene in mammary carcinomas
Aneuploidy	+12 in chronic lymphocytic leukemia +8 in ANLL, blast phase of CML, polyps of colon, preleukemia +15 in murine T-cell leukemias −15 in Syrian hamster tumors induced by transfection of v-Ha-*ras* and v-*myc*

The mechanisms of carcinogen-induced activation of oncogenes have been elucidated, and the implications of these findings are highly important for the risk assessment of chemicals. This point can be illustrated by three examples: (a) Carcinogen-induced point mutations, resulting in activation of a *ras* oncogene, have been demonstrated in carcinogenesis of skin (*4*), mammary gland (*5*), and liver (*6,7*). In these model systems, the data support the conclusion that these point mutations are the critical changes in the initiation of these tumors. These findings provide strong experimental evidence for using the linear dose-response curves observed in mutagenesis studies for carcinogen risk assessment. (b) Elucidation of oncogene activation by other genetic changes (Table 3) such as chromosome rearrangements and gene amplification provides a theoretical framework for the use of these end points in risk assessment. (c) The observations that normal cells are not neoplastically transformed by a single oncogene, but rather, require two or more cooperating oncogenes, support a multistep model of carcinogenesis (*47,51*) and have significant implications for risk assessment of chemicals. Since at least two protooncogenes have to be activated for a tumor cell to arise and these activation events may occur by different genetic mechanisms, it is not surprising that a single carcinogen-DNA adduct or toxicological end point does not always correlate with carcinogenic potency of chemicals.

Table 4. Lines of evidence for tumor suppressor genes.

Cell hybrids
Tumorigenicity is generally suppressed in hybrids between normal and tumorigenic cells
Reexpression of tumorigenicity of these cell hybrids is associated with loss of specific chromosomes
Transfer of a single, normal chromosome via microcell transfer methods can suppress tumorigenicity of human tumor cells
Genetic predispositions to cancer
Dominantly inherited cancer susceptibilities in humans (e.g., retinoblastoma and Wilms' tumor) involve a germline, heterozygous mutation that becomes homozygous or hemizygous in tumor tissue. Recently, a candidate for the retinoblastoma gene has been cloned by several groups
Dominant inheritance of susceptibility to renal and mammary carcinoma in the rat may involve tumor suppressor genes
More than 20 recessive-lethal mutants of *Drosophilia melanogaster* are associated with predispositions to tissue-specific malignant neoplasms
Hybrid fish between ornamental platyfish (*Xiphophorus maculatis*) and ornamental swordtail (*Xiphophorus helleri*) have increased incidence of tumors that appear to result from inheritance of a tumor gene (oncogene) and elimination of regulatory (tumor suppressor) gene
RFLP analyses
Analyses of restriction-fragment-length polymorphism of nucleic acid probes show that heterozygous alleles on specific chromosomes frequently become homozygous in certain human tumors
Chromosome deletions
Nonrandom chromosome deletions or losses are observed in specific types of cancers

Oncogenes have received a great deal of attention since their discovery, and the importance of their role in carcinogenesis has been adequately demonstrated (*41,42,51*). However, considerable evidence exists that tumor suppressor genes play an equally important role in neoplastic development (Table 4). Recent data from our laboratory have shown that in the multistep process of chemical carcinogen-induced neoplastic transformation of Syrian hamster embryo cells in culture, the loss of a tumor suppressor gene function is an essential step (Fig. 2); without the loss of this gene activity, multiple oncogenes are unable to neoplastically transform the cells (*52–54*).

One important distinction between oncogenes and tumor suppressor genes is the differences in the mechanisms by which carcinogens act upon these genes (Table 2). Protooncogenes have to be activated to influence carcinogenesis. This activation event may be a qualitative or quantitative alteration caused by point mutations, chromosome rearrangements, or gene amplification (Table 3). In contrast, tumor suppressor genes have to be inactivated in order for the tumorigenic phenotype to be expressed. This inactivation may result from chromosome loss, chromosome or gene deletion, recombination, gene conversion, or possibly point mutation (*49,50*). The mechanisms of action and the dose responses of carcinogens in inducing the different types of genetic changes required for the activation of protooncogenes or inactivation of tumor suppressor genes

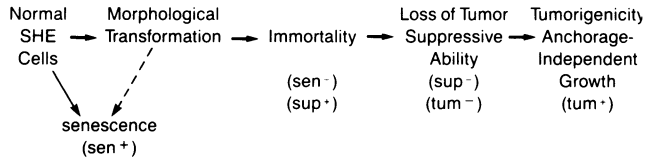

FIGURE 2. Neoplastic progression of Syrian hamster cells.

may vary considerably. These multiple genetic changes, along with the influence of tumor promoting effects in carcinogenesis, are likely to account for the difficulties in making simple correlations between carcinogenic activity and any one property of a carcinogen.

Conclusions

Chemical carcinogenesis is generally a multistep process. Not only does this process involve the stages of initiation and promotion, but also heritable alterations in multiple genes must occur during neoplastic progression. The genes involved in these multiple stages appear to represent two distinct classes: protooncogenes, which must be activated or expressed inappropriately, and tumor suppressor genes, which must be inactivated or lost. The mechanisms of activation or inactivation of these genes may be quite distinct. Little is known about the dose-responses of chemically induced genetic changes other than point mutations. The dose-response for the carcinogenic activity response of a chemical will be a composite of its effects on different genetic changes. In addition, epigenetic properties of the chemical that influence the clonal proliferation of the initiated cells (i.e., tumor promotion) may also be important in determining carcinogenic potency. When chronic exposure is involved, few chemicals, if any, will affect only one stage in the multistep carcinogenic process. Thus, the complex interaction of multiple genetic and epigenetic factors in carcinogenesis should caution against simplistic models of carcinogen risk assessment on the basis of a single genotoxic or epigenetic mechanism of action.

NOTE ADDED IN PROOF: An excellent review of this subject, entitled "Multistage Carcinogenesis: Implications for Risk Assessment," by H. Yamasaki is in press in *Cancer Metastasis Reviews*. Important insights concerning dose and time responses of complete carcinogens and promoters are discussed in this review, which is highly recommended.

REFERENCES

1. Barrett, J. C. Mechanisms of Environmental Carcinogenesis, Vol. II. CRC Press, Boca Raton, FL, 1987.
2. Pitot, H. C., Goldsworthy, T., and Moran, S. The natural history of carcinogenesis: Implication of experimental carcinogenesis in the genesis of human cancer. J. Supramol. Struct. Cell. Biochem. 17: 133–146 (1981).
3. Barbacid, M. Mutagens, oncogenes and cancer. Trends Genet. 2: 188–192 (1986).
4. Quintanilla, M., Brown, K., Ramsden, M., and Balmain, A. Carcinogen-specific mutation and amplification of Ha-*ras* during mouse skin carcinogenesis. Nature 322: 78–80 (1986).

5. Zarbl, H., Sukumar, S., Arthur, A. V., Martin-Zanca, D., and Barbacid, M. Direct mutagenesis of Ha-*ras*-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats. Nature 315: 382–385 (1985).
6. Wiseman, R. W., Stowers, S. J., Miller, E. C., Anderson, M. W., and Miller, J. A. Activating mutations of the c-Ha-*ras* proto-oncogene in chemically induced hepatomas of the male B6C3F_1 mouse. Proc. Natl. Acad. Sci. (U.S.) 83: 5825–5829 (1986).
7. Reynolds, S., Stowers, S. J., Patterson, R. M., Maronpot, R., Aaronson, S., and Anderson, M. Activated oncogenes in spontaneous and chemical associated mouse liver tumors: Implications for risk analysis. Science 237: 1309–1316 (1987).
8. Berenblum, I., and Haran-Ghera, N. A quantitative study of the systemic initiating action of urethane (ethyl carbamate) in mouse skin carcinogenesis. Br. J. Cancer 11: 77–84 (1957).
9. Dahl, G. A., Miller, E. C., and Miller, J. A. Comparative carcinogenicities and mutagenicities of vinyl carbamate, ethyl carbamate and ethyl N-hydroxyethylcarbamate. Cancer Res. 40: 1194–1203 (1980).
10. Boutwell, R. The function and mechanism of promoters of carcinogenesis. CRC Crit. Rev. Toxicol. 2: 419–443 (1974).
11. Astrup, E. G., Iversen, O. H., and Elgjo, K. The tumorigenic and carcinogenic effect of TPA (12-O-tetradecanoylphorbol-13-acetate) when applied to the skin of Balb/cA mice. Virchows Arch. B. Cell Pathol. 33: 303–304 (1980).
12. Fujiki, H., Suganuma, M., Matsukura, N., Sugimura, T., and Takayama, S. Teleocidin from Streptomyces is a potent promoter of mouse skin carcinogenesis. Carcinogenesis 3: 895–898 (1982).
13. Hennings, H., Shores, R., Mitchell, P., Spangler, E., and Yuspa, S. Induction of papillomas with a high probability of conversion to malignancy. Carcinogenesis 6: 1607–1610 (1985).
14. O'Connell, J. F., Klein-Szanto, A. J. P., DiGiovanni, D. M., Fries, J. W., and Slaga, T. J. Enhanced malignant progression of mouse skin tumors by the free-radical generator benzoyl peroxide. Cancer Res. 46: 2863–2865 (1986).
15. Shubik, P. The growth potentialities of induced skin tumors in mice. The effects of different methods of chemical carcinogenesis. Cancer Res. 10: 713–717 (1950).
16. Burns, F., Albert, R., Altshuler, B., and Morris, E. Approach to risk assessment for genotoxic carcinogenesis based on data from the mouse skin initiation-promotion model. Environ. Health Perspect. 50: 309–320 (1983).
17. Scribner, J. D., Scribner, N. K., McKnight, B., and Mottet, N. K. Evidence for a new model of tumor progression from carcinogenesis and tumor promotion studies with 7-bromomethylbenz[a]anthracene. Cancer Res. 43: 2034–2041 (1983).
18. Hennings, H. Tumor promotion and progression in mouse skin. In: Mechanisms of Environmental Carcinogenesis, Vol. I (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 59–71.
19. Barrett, J. C. Tumor promotion and tumor progression. In: Carcinogenesis—A Comprehensive Survey, Vol. 8 (M. J. Mass, D. G. Kaufman, J. M. Siegfried, V. E. Steele, and S. Nesnow, Eds.), Raven Press, New York, 1984, pp. 423–429.
20. O'Connell, J. F., Klein-Szanto, A. J. P., DiGiovanni, D. M., Fries, J. V., and Slaga, T. J. Malignant progression of mouse skin papillomas treated with ethylnitrosourea, N-nitro-N-nitroso-guanidine, or 12-O-tetradecanoylphorbol-13-acetate. Cancer Let. 30: 268–274 (1986).
21. Hennings, H., Shores, R., Wenk, M., Spangler, E., Torane, R., and Yuspa, S. Malignant conversion of mouse skin tumors is increased by tumor initiators and unaffected by tumor promoters. Nature 304: 67–69 (1983).
22. Hennings, H., and Yuspa, S. Two-stage tumor promotion in mouse skin: An alternative interpretation. J. Natl. Cancer Inst. 74: 735–740 (1985).
23. Aldaz, C. M., Conti, C. J., Klein-Szanto, A. J. P. and Slaga, T. J. Progressive dysplasia and aneuploidy are hallmarks of mouse skin papillomas: Relevance to malignancy. Proc. Natl. Acad. Sci. (U.S.) 84: 2029–20321 (1987).
24. Conti, C. J., Aldaz, C. M., O'Connell, J. F., Klein-Szanto, A. J. P., and Slaga, T. J. Aneuploidy, an early event in mouse skin tumor development. Carcinogenesis 7: 1845–1848 (1986).
25. Farber, E., Rotstein, J. B., and Eriksson, L. C. Cancer development as a multistep process: Experimental studies in animals. In: Mechanisms of Environmental Carcinogenesis, Vol. II (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 1–19.
26. Potter, V. R. A new protocol and its rationale for the study of initiation and promotion of carcinogenesis in rat liver. Carcinogenesis 2: 1375–1379 (1981).
27. Foulds, L. Neoplastic Development, Vol. I. Academic Press, London, 1969.
28. Kaldor, J. M., and Day, N. E. Interpretation of epidemiological studies in the context of the multi-stage model of carcinogenesis. In: Mechanisms of Environmental Carcinogenesis, Vol. II (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 21–57.
29. Carcinogenesis of Arsenic and Arsenic Compounds. IARC Monograph on Evaluation of Carcinogenic Risk to Humans 23: 137–141 (1980).
30. IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans. (Suppl.) 4: 50–51 (1982).
31. Ishinishi, N., Yamamoto, A., Hisanga, A., and Inamasu, T. Tumorigenicity of arsenic trioxide to the lung in Syrian golden hamster by intermittent instillations. Cancer Let. 21: 141–147 (1983).
32. Rudnay, P., and Borzsonyi, M. The tumorigenicity effect of treatment with arsenic trioxide. Magyar Onkol. 25: 73–77 (1981).
33. Pershagen, G., Nordberg, G., and Bjorklund, N. Carcinomas of the respiratory tract in hamsters given arsenic trioxide and/or benzo(a)pyrene by pulmonary route. Environ. Res. 34: 227–241 (1984).
34. Boutwell, R. K. A carcinogenicity evaluation of potassium arsenite and arsanillic acid. J. Agric. Food Chem. 11: 381–384 (1963).
35. Baroni, C., Esch van, G. J., and Saffiotti, V. Carcinogenesis tests of two inorganic arsenicals. Arch. Environ. Health 7: 668–678 (1963).
36. Brown, C. C., and Chu, K. C. Implications of the multistage theory of carcinogenesis applied to occupational arsenic exposure. J. Natl. Cancer Inst. 70: 455–463 (1983).
37. Barrett, J. C., Lamb, P. W., Lee, T. C., Oshimura, M., Tanaka, N., and Tsutsui, T. Possible roles of gene duplication and amplification in a multiple step model of cell transformation. In: The Role of DNA Amplification in Carcinogenesis, Accomplishments in Oncology (H. ZurHausen and J. Schlehofer, Eds.), J. B. Lippincott, Philadelphia, in press.
38. Schwab, M. Enhanced expression of the human gene N-*myc* consequent to amplification of DNA may contribute to malignant progression of neuroblastomas. Proc. Natl. Acad. Sci. (U.S.) 81: 4940–4944 (1984).
39. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E., and Bishop, J. M. Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. Science 224: 1121–1124 (1984).
40. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. I., Ullrich, A., and McGuire, W. L. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235: 177–182 (1987).
41. Bishop, J. M. The molecular genetics of cancer. Science 235: 305–322 (1987).
42. Weinberg, R. A. The action of oncogenes in the cytoplasm and nucleus. Science 230: 770–776 (1985).
43. Barrett, J. C. A model for neoplastic development: Role of genetic and epigenetic changes. In: Mechanisms of Environmental Carcinogenesis, Vol. II (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987.
44. Knudson, A. G., Jr. Hereditary cancer, oncogenes, and antioncogenes. Cancer Res. 45: 1437–1443 (1985).
45. Weismann, B. E. Suppression of tumorigenicity in mammalian cell hybrids. In: Mechanisms of Environmental Carcinogenesis, Vol. I (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987, pp. 31–45.
46. Stanbridge, E. J. Suppression of malignancy in human cells. Nature 260: 17–20 (1976).
47. Sager, R. Genetic suppression of tumor formation: A new frontier in cancer research. Cancer Res. 46: 1573–1580 (1986).
48. Gateff, E. Malignant neoplasms of genetic origin in *Drosophila melanogaster*. Science 200: 1448–1459 (1978).
49. Cavenee, W. K., Dryja, T. P., Phillips, R. A., Benedict, W. F.,

Godbout, B. L., Gallie, B. L., Murphree, A. L., Stong, L. C., and White, R. L. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305: 779–784 (1983).
50. Murphree, A. L., and Benedict, W. F. Retinoblastoma: Clues to human oncogenesis. Science 223: 1028–1033 (1984).
51. Barrett, J. C., and Fletcher, W. F. Cellular and molecular mechanisms of multistep carcinogenesis in cell culture models. In: Mechanisms of Environmental Carcinogenesis, Vol. II (J. C. Barrett, Ed.), CRC Press, Boca Raton, FL, 1987.
52. Koi, M., and Barrett, J. C. Loss of tumor suppression function during chemically induced neoplastic progression of Syrian hamster embryo cells. Proc. Natl. Acad. Sci. (U.S.) 83: 5992–5996 (1986).
53. Oshimura, M., Gilmer, T. M., and Barrett, J. C. Nonrandom loss of chromosome 15 in Syrian hamster tumors induced by v-Ha-*ras* plus v-*myc* oncogenes. Nature 316: 636–639 (1985).
54. Barrett, J. C., Oshimura, M., and Koi, M. Role of oncogenes and tumor suppressor genes in a multistep model of carcinogenesis. Symp. Fundam. Cancer Res. 32: in press.