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Review Hypothesis

A Unified Theory for the Development of Cancer

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It is postulated that cancer is the result of genetic and epigenetic changes that occur mainly in stem (precursor) cells of various cell types. I propose that there are three classes of genes which are involved in the development of cancer. These are: Class I, II and III oncogenes. The classification is based on the way the oncogene acts at the cellular level to further the development of cancer. Genetic changes, that is point mutations, deletions, inversions, amplifications and chromosome translocations, gains or losses in the genes themselves or epigenetic changes in the genes (e.g. DNA hypomethylation) or in the gene products (RNA or protein) are responsible for the development of cancer. Changes of oncogene activity have a genetic or epigenetic origin or both and result in quantitative or qualitative differences in the oncogene products. These are involved in changing normal cells into the cells demonstrating a cancer phenotype (usually a form of dedifferentiated cell) in a multistep process. There are several pathways to cancer and the intermediate steps are not necessarily defined in an orderly fashion. Activation of a particular Class I or II oncogene and inactivation of a Class III oncogene could occur at any step during the development of cancer. Most benign or malignant tumors consist of a heterogeneous mixture of dedifferentiated cells arising from a single cell.

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INTRODUCTION

Epidemiological analyses suggest that human cancer is caused by a combination of environmental and genetic factors. Thus it is now recognised that cancer rates are influenced by the environment (pollutants, combustive chemical exposure, viruses), lifestyle (smoking, alcoholic beverages) and diet (presence or absence of anti-oxidant factors, vitamins, fiber or fats) (1). It is thought that 90% of cancers are due to environmental factors. The remaining 10% of all cancers can probably be attributed to a hereditary component and to spontaneous mutations.

Molecular biological studies now emphasise the importance to human cancer of changes in the quantitative or qualitative expression of particular classes of genes collectively known as oncogenes (for reviews see refs. 2 and 3). Activation of these oncogenes is the result primarily of mutations induced by chemicals, radiation or viruses. However, there is also evidence that epigenetic changes influence carcinogenesis (4, 5).

Several theories have been proposed to explain the development of cancer (6–12). Some, such as the oncogene theory of Huebner and Todaro (8), have made significant contributions to our understanding of the disease. However, the great advances in our knowledge of the cancer cell in the last few years have made it clear that the situation is very complicated. Recent progress in cloning and detailed characterization of cellular oncogenes (for reviews see refs. 2 and 3), and the development of efficient gene transfer methods (for a review see ref. 13), has allowed the formulation of new ideas and concepts about cancer (2, 14, 15, 16).

In this paper I present a theory for the development of cancer taking into account the advances made in the last 10 years of research in cancer.

A UNIFIED THEORY OF CANCER

Definitions

Mutation is defined here as a heritable genetic change in the cell. It is a change in the genetic content of the cell and it can be a point mutation, a deletion or insertion of one or more nucleotides, amplification of a segment of DNA, inversion or a chromosome gain, loss or translocation. The essential point about a mutation is that the genetic material of the cell is altered, not simply its expression.

An epigenetic change is an alteration in gene expression without any change in the DNA sequence or genetic content of the cell. Although genetic changes can be relatively easily identified, there are no appropriate detection systems for epigenetic changes with the exception of methylation.

An initiated cell is a mutated cell with the potential to become a tumor or a malignant cell.

A benign tumor is a local outgrowth of the initiated cell. When a benign tumor is destined to become malignant it is called premalignant. A malignant tumor or cancer is a tumor which has the ability to invade tissues and spread to distant sites. The term

neoplasia (new growth) is used to encompass the whole range of benign and malignant cell behavior. When necessary a tumor will be qualified as benign or malignant.

The concepts of dominance and recessiveness are complicated issues and beyond the scope of this article. Because of these complications no attempt will be made to classify the oncogenes in these terms. For most of them however, their behavior in terms of dominance or recessiveness is totally unknown.

The cellular genes that influence the development of cancer either directly or indirectly are termed oncogenes. These oncogenes are cancer related genes and they can be divided into three classes. These are Class I, Class II and Class III.

Class I oncogenes act directly on a cell to give a transformed phenotype. They were discovered through experiments of gene transfer of cancer markers (17–19) and through retrovirus studies (for a review see ref. 2). The expression of transfected Class I oncogenes in recipient cells implies that they act dominantly at the cellular level at least *in vitro*. However, *in vivo* this may not be the case (20). So far, more than 30 such genes are known but their number may be higher (2, 3). They can be subdivided into those found in retroviruses (Table 1) and those not found in retroviruses (Table 2).

Class II oncogenes affect the transformed phenotype of the cell indirectly, that is through the action of a Class I or Class III oncogene. More than 20 such genes are known (Tables 3 and 4). They are associated with the conditions xeroderma pigmentosum (at least 9 complementation groups), ataxia telangiectasia (at least 4 complementation groups), Bloom's syndrome, Fanconi's anemia and Cockayne's syndrome. These conditions have lesions in DNA repair or exhibit chromosome instability and they behave recessively in a Mendelian fashion (for reviews see ref. 21,

Table 1. Class I oncogenes found in retroviruses

Gene	Origin
1. src	chicken
2. fps	chicken
3. yes	chicken
4. ros	chicken
5. ski	chicken
6. erbA	chicken
7. erbB	chicken
8. myc	chicken
9. myb	chicken
10. mht/mil	chicken
11. ets	chicken
12. rcl	turkey
13. abl	mouse
14. fos	mouse
15. mos	mouse
16. raf	mouse
17. Ha-ras	rat
18. Ki-ras	rat
19. fes	cat
20. fgr	cat
21. fms	cat
22. kit	cat
23. sis	monkey

Table 2. Class I oncogenes not found in retroviruses

Gene	Origin
1. neu	Rat (neuro-glioblastoma)
2. N-ras	Human (neuroblastoma/fibrosarcoma)
3. N-myc	Human (neuroblastoma)
4. p53	Mouse, human
5. Onc D	Human (colon Ca.)
6. Onc E	Human (osteosarcoma cell line)
7. met	Human (osteosarcoma cell line)
8. int1	Mouse (insertion site for MMTV)
9. int2	Mouse (insertion site for MMTV)
10. piml	Mouse (insertion site for MuLV)
11. bcl1*	Human (rearrangement in translocation)
12. bcl2*	Human (rearrangement in translocation)
13. a-TGF*	Rat (transforming growth factor)
14. Mlvi-1*	Rat (insertion site for MoMSV)
15. Mlvi-2*	Rat (insertion site for MoMSV)
16. Mlvi-3*	Rat (insertion site for MoMSV)

* These have not yet been shown to have a transforming activity.

Table 3. Diseases in which Class II oncogenes may be involved

Disease	Origin
1. Xeroderma pigmentosum (9 complementation groups)	Human
2. Bloom's syndrome	Human
3. Ataxia telangiectasia (4 complementation groups)	Human
4. Fanconi's anemia	Human
5. Cockayne's syndrome	Human
6. Immune deficiency syndromes	Human
7. Peutz-Jeghers syndrome	Human
8. Cowden's syndrome	Human
9. Albinism	Human
10. Variations in estrogen metabolism	Human
11. Down's syndrome	Human
12. Klinefelter's syndrome	Human

Table 4. Class II oncogenes

Gene	Origin
1. Mutator gene	Hamster
2. X-LOR (trans-activator)	Human (HTLVI)
3. Aryl hydrocarbon hydroxylase (AHH)	Human

22). Mutator genes (23), or related genes that affect the metabolism of DNA, carcinogen or hormones also fall into this group (Table 3). One such gene is the aryl hydrocarbon hydroxylase (AHH) which is involved in the metabolism of carcinogens (24). Also included in the same class are the genes that are involved in the immune deficiency syndromes, disturbances of tissue organization like the hamartomatous syndromes (Peutz-Jeghers and Cowden) and the X-LOR gene of the human retrovirus HTLV I (25). Finally, constitutional chromosomal abnormalities such as Down's and Klinefelter's syndromes have an increased risk for cancer (21, 26) and the genes responsible also fall into this class (Table 3). Thus, the ultimate result of having an activated Class II oncogene, is predisposition to cancer. It should also be recognised that the distinction between Class I and Class II oncogenes may be blurred at the edges. For example genes like *myc*, *erbA* and X-LOR may function both as Class I and Class II oncogenes, and in xeroderma pigmentosum the inactivation of a DNA repair enzyme could be regarded as either a Class III or a Class II oncogene defect.

Class III oncogenes are genes, the absence of whose activity contributes to the development of cancer (Tables 5 and 6). In two conditions, retinoblastoma (27, 29) and Wilm's tumor (30), the genes have been studied in some detail. They act recessively at the cellular level, but behave dominantly in a Mendelian fashion and when inactivated they predispose to cancer. Included in this group are the cancer genes in pediatric predisposition syndromes such as the Beckwith-Wiedemann syndrome, neuroblastoma, neurofibromatosis and medullary carcinoma of the thyroid (Table 5;

Table 5. Diseases in which Class III oncogenes may be involved

Disease	Origin
1. Retinoblastoma	Human
2. Wilms' tumor	Human
3. Neuroblastoma	Human
4. Beckwith-Wiedemann syndrome	Human
5. Neurofibromatosis	Human
6. Medullary carcinoma of the thyroid	Human
7. Adenomatosis of colon and rectum (ACR)	Human
8. Familial breast cancer	Human

Table 6. Class III oncogenes

Gene	Origin
1. β-Interferon	human
2. β-Transforming growth factor (β-TGF)	rat
3. Fibroblast growth regulator (FGR-S)	mouse
4. Hepatic proliferation inhibitor (HPI)	rat
5. Bovine glycopeptide inhibitor (BCSG)	bovine

for a review, see ref. 31). Finally, this class also includes the genes for the adenomatosis of the colon and rectum (ACR), familial breast cancer (for reviews see refs. 21, 32) and those coding for negative regulators of cell growth such as the β-tumor growth factor (β-TGF), β-interferon, fibroblast growth regulator (FGR-S), hepatic proliferation inhibitor (HPI) and bovine glycopeptide inhibitor (BCSG) (for a review see ref. 33). It is postulated that there are more than 100 of this type of genes, as many as the different forms of cancer. However, only the first two examples in Table 6 (retinoblastoma and Wilm's tumor) have clear-cut genetic evidence to assign them to the Class III oncogene family. The other examples are conditions or genes which are believed to have a similar behavior.

The Model

I present a model for the development of cancer (Fig. 1), the main features of which are the following. A normal cell is converted to an initiated cell which carries one critical mutation (initiation stage) which confers to it a proliferative advantage after the action of promoters (promotion stage). Benign tumors are considered to be a heterogeneous cell population with individual cells carrying one or more additional mutations or epigenetic changes in addition to the initiating mutation. Both benign and malignant tumors are therefore clonal with respect to the progenitor cell but

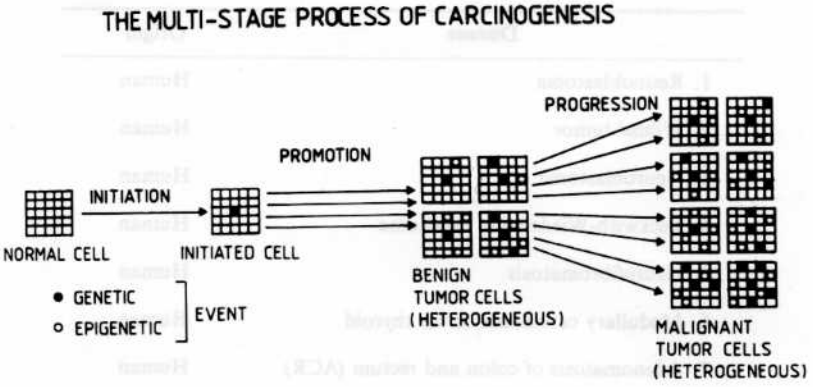

Fig. 1. The development of malignancy. The normal cell is represented by a square divided into 25 small squares each one denoting a point wheee a genetic or epigenetic change could occur. See text for details.

heterogeneous with respect to acquisition of additional mutations. The tumor cells can be converted to malignant cells after the action of progressors (progression stage). Malignant tumors are a heterogeneous cell population with individual cells carrying one or more mutations or epigenetic changes in addition to those present in their parent tumor cells.

The Class I and Class II oncogenes can be activated and the Class III oncogenes can be inactivated in any of these stages. Oncogenes cannot be rigidly classified into "immortalization" or "tumorigenic" groups (34) in this model since they can act on any of these stages. However, in some cases preferences in oncogene activation may be occurring at a particular stage of a particular tumor.

Although the above processes of initiation, promotion and progression are involved in the conversion of a normal to a cancer cell, it should be pointed out that cells at all three stages can be obtained at very low frequencies by spontaneous genetic or epigenetic changes.

It is also important to note here that while an initiated and a tumorigenic stage are essential to cancer development, not all initiated or tumorigenic cells necessarily develop into malignant cells.

RESULTS AND DISCUSSION

Multistage Carcinogenesis

The evidence that a number of distinct steps is involved in the formation of a malignant cell comes from studies of experimental cancer induction in animals (35), *in vitro* cell transformation assays (36), histopathological (7) and biochemical (37) analyses of tumors and from epidemiological studies in man (38).

Circumstantial evidence for the role of mutation in the genesis of cancer came from the following observations. 1. Most carcinogens are mutagens (39). 2. There is a relationship between the induction of mutations in cells in culture and the appearance of the transformed phenotype (40). 3. The existence of specific chromosomal abnormalities associated with certain cancers (41). More direct evidence comes from: 4. A predisposition to certain cancers which is inherited in a clear Mendelian fashion, e.g. retinoblastoma (42) or xeroderma pigmentosum (43), and 5. The isolation and characterization of transforming oncogenes (for reviews, see refs. 2, 3).

Whereas some polygenic disorders exist in man, many disorders and traits have been found to be determined by single genes. In the fourth edition of Mendelian Inheritance in Man (44), 2336 certain or possible single gene traits are listed. Of these 200 (9%) have neoplasia as a sole feature, an occasional finding, or a rare complication (45). Therefore, it is reasonable to assume that the potential number of Class I, Class II and Class III oncogenes is not likely to exceed 9% of all human genes. This is because mutations of many genes (perhaps most) may give rise to neither cancer nor any detectable Mendelian trait or syndrome.

Studies on polygenic disorders with an increased risk of cancer can be divided into ethnic differences in cancer incidence and family studies. Both these types of analyses have provided evidence for the existence of multiple genetically determined traits

affecting cancer risk. Colonic carcinoma patients may be predisposed to cancer as a result of at least eleven different inherited traits (21, 46).

Oncogenes and Their Involvement in Carcinogenesis

There is now good evidence for the existence of these genes in the human genome and their importance in human cancer. Following the initial experiments on the transfer of cancer markers via metaphase chromosomes (17, 18) using the calcium phosphate technique of Graham and Van der Eb (47), an explosion in the field of oncogene research has occurred in the past 10 years which has resulted in the isolation and characterization of Class I oncogenes and their products (for reviews see refs. 2, 3). One such group of Class I oncogenes, the *ras* family, which are the cellular homologues of the retroviral Harvey and Kirsten *ras* oncogenes, are activated by mutation of the structural gene in at least 20% of human tumors (20), and in 70% of chemically-induced tumors in several animal model systems (48–50). Taking into account other forms of oncogenic activation, e.g. elevated expression of the normal gene by regulatory mutations or by gene amplifications, the percentage of human tumors carrying activated *ras* oncogenes may be higher (51–54). Given the occurrence of tumor heterogeneity (55) and the multiple choice for oncogene activation in the multistage pathway of cancer (16), this theory fits in well with these findings.

Progress has been slower on the molecular characterization of Class II and Class III oncogenes. However, cloning and partial characterization of the first DNA repair gene has been reported (56) and the retinoblastoma RB-1 gene has been mapped to band q14 of human chromosome 13 (28, 29).

The role of individual oncogenes in multistage carcinogenesis is not well understood at present. However, there is strong evidence that the *ras* and *myc* oncogenes can be activated at all three stages of carcinogenesis. Thus Ha-*ras* is activated at early stages of carcinogenesis (20, 49, 51, 53, 57, 58), intermediate (57, 59, 60), as well as late (61–63). Similarly, the *myc* oncogene has been implicated in early (64), intermediate (16, 59, 65), and late (66) stages. Thus oncogenes cannot be strictly placed into rigid categories (34) according to the steps in which they might act during carcinogenesis (16). The apparent lack of tissue specificity of some oncogenes, e.g. *ras* oncogenes have been found to be activated in a variety of tissues such as bladder, colon, gall bladder, liver, lung, pancreas, etc. (for a review see ref. 20), is another emerging principle consistent with the model presented here.

Genetic Mechanisms of Carcinogenesis

A large variety of mechanisms for the activation of oncogenes have been uncovered (Fig. 2) and their main features are summarised below.

Transduction

Transduction is the process by which a transforming retrovirus carrying an activated oncogene infects a cell and converts it into a cancer cell. This has been shown to occur frequently in tumors induced by feline retroviruses where a naturally

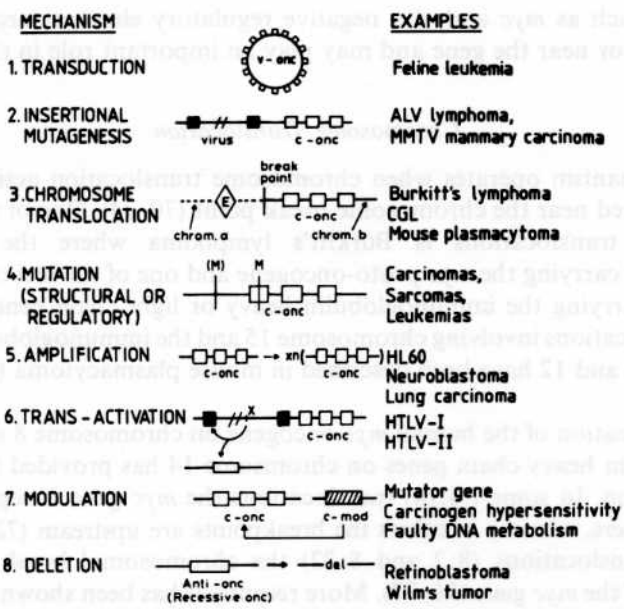

Fig. 2. Genetic mechanisms for carcinogenesis. See text for details. V-onc, viral oncogene; c-onc, cellular oncogene; E, enhancer; M, mutation; Anti-onc, antioncogene; del, deletion.

occurring slow transforming retrovirus recombines with cellular proto-oncogenes. The transduced oncogenes in these cases behave abnormally (67, 68). The oncogenes present in transforming retroviruses belong to Class I.

Insertional Mutagenesis

In insertional mutagenesis, the slow transforming retrovirus, mainly the long terminal repeat (LTR) sequences containing the transcriptional enhancer and promoter elements are inserted next to a cellular oncogene, e.g. c-*myc*, and activate its transcription (69). Insertion can occur either upstream or downstream of the cellular oncogene. This mechanism was originally named the promoter insertion hypothesis because transcription initiated at the viral promoter. However with the discovery of a transcriptional enhancer in the LTR and the demonstration that in many cases the virus does not provide a promoter for the proto-oncogene, this terminology may not be appropriate and enhancer insertion has been used to describe this type of mutagenesis (for a review see ref. 2).

Recent transfection experiments *in vitro* have shown that when a retroviral LTR is covalently linked to the *ras* cellular oncogene and transfected into rodent cells, oncogene activation occurs and produces cell immortalization (57) or tumorigenic conversion (16, 57, 60).

So far, insertional mutagenesis has been observed and studied experimentally only with Class I oncogenes, but it is possible that a similar mechanism occurs with Class II oncogenes (activation) and Class III oncogenes (inactivation). For some Class

I oncogenes such as *myc* and *fos*, negative regulatory elements are thought to be located within or near the gene and may play an important role in their expression.

Chromosome Translocation

This mechanism operates when chromosome translocation activates a cellular oncogene located near the chromosome break point (70–74). One of the best studied chromosomal translocations is Burkitt's lymphoma where the long arm of chromosome 8 carrying the *myc* proto-oncogene and one of the loci of chromosomes 14, 2 or 22, carrying the immunoglobulin heavy or light chain genes are involved. Similar translocations involving chromosome 15 and the immunoglobulin gene loci on chromosome 6 and 12 have been described in mouse plasmacytoma (for a review see ref. 70).

The localization of the human *myc* oncogene on chromosome 8 and its fusion to immunoglobulin heavy chain genes on chromsome 14 has provided important clues for its activation. In some t(8:14) translocations the *myc* gene is separated from its natural promoters, whereas in others the breakpoints are upstream (72, 75). In almost all variant translocations (8:2 and 8:22) the chromosomal break points are far downstream of the *myc* gene (76, 77). More recently it has been shown that truncation of exon I from the *myc* gene results in prolonged *myc* RNA stability (78, 79). Therefore post-transcriptional mechanisms may well operate in this system. Although only Class I oncogenes such as *myc* or *abl* (80) are known to be activated by chromosome translocations, theoretically at least it is possible that Class II and Class III oncogenes could also be activated or inactivated respectively by similar mechanisms.

Mutation

The first demonstration of oncogene activation by point mutation was obtained from studies with the Ha-*ras* 1 oncogene in the T24 human bladder carcinoma cell line (81–83). Subsequent studies of human tumors showed that *ras* activation was produced by single amino acid substitutions at positions 12 or 13 or 61 of the *ras p*21 product (84, 85). *In vitro* mutagenesis studies have shown that amino acid substitutions at positions 59 and 63 can also activate the Ha-*ras* gene (86, 87). Moreover, chemical carcinogens can activate the Ha-*ras* oncogene in the rat by causing a mutation in codon 12 (48). These studies suggest that activation can occur by mutation of a small number of codon positions in the structural Class I oncogene. Activation by mutation in Class II oncogenes and inactivation in Class III oncogenes may occur in a similar manner.

Amplification

Amplification of several oncogenes including *myc*, N-*myc*, *myb*, Ki-*ras*, *abl* and *erb B* has been found in several human cancer cell lines and in fresh human tumors (54, 58). The amplified oncogene is often associated with double minute chromosomes (DMs) or homogeneously staining regions of chromosomes (HSRs). Amplification could occur for any of the Class I, II or III oncogenes.

Trans-activation

The human retrovirus HTLV I encodes a gene, X-LOR, whose product acts in trans to produce transcription from the viral LTR promoter and probably from other cellular promoters (25, 89). Similarly the product of the adenovirus EIA gene activates certain cellular genes and represses others (90, 91). Trans-activation is a property of some Class II oncogenes. However, in contrast to the HTLVI X-LOR and adenovirus ElA genes, no cellular genes are known to function in a similar fashion.

Modulation

Several genes can be placed in this category. Their activated forms could cause the cells sensitivity to UV light or chromosomal instability (i.e. xeroderma pigmentosum or ataxia telangiectasia (for a review see ref. 22) and increased mutation frequencies and rates of other genes (mutator gene) (23). Modulation is a property of some Class II oncogenes. Modulator genes can activate a Class I or Class II oncogene.

Deletion

This type of gene inactivation is particularly associated with oncogenes which predispose to cancer but behave in a recessive cellular manner and the cancer phenotype appears when both alleles are inactivated. Their presence has been well documented in *Drosophila melanogaster* in which at least 24 recessive genes have been identified. Defects in these genes cause tissue specific tumors (92).

There is increasing evidence that human retinoblastoma is caused by a similar type of mechanism (28, 29). A gene (RB-1) associated with human retinoblastoma maps to band q14 of human chromosome 13. Loss of activity is thought to arise by a variety of mechanisms such as non-disjunctional loss of a normal chromosome 13, duplication of a single mutated chromosome 13, loss of the normal mitotic recombination, or a gene conversion event (28). These results strongly support the theory proposed by Knudson (42) that this particular tumor arises by two mutational steps resulting in homozygosity of mutations at the RB-1 locus. Although a deletion will inactivate a Class III oncogene, the loss of whose activity contributes to cancer, as demonstrated in retinoblastoma and similarly found in Wilms' tumor (30), it is conceivable that deletions could activate Class I and Class II oncogenes.

Epigenetic Mechanisms of Carcinogenesis

Tumor Promotion and Progression

The major effect of tumor promoters is the specific expansion of the initiated cell population in a target tissue. The promotion phase is initially reversible, later becoming irreversible.

Studies using cell culture systems have suggested that the primary action of tumor promoters takes place at the cell membrane (for a review see ref. 93).

The mouse skin tumor promoters have different potencies. Some like the TPA (12-

0-tetradecanoylphorbol-13-acetate) are strong, others like benzoyl peroxide are moderate and some others like iodoacetic acid are weak in their promotion effect (94). It is presently thought that tumor promoters do not bind covalently to DNA and are not mutagenic, they exert their effect on the initiated cell by causing important epigenetic changes. Apart from causing morphological and biochemical changes, the phorbol esters and other tumor promoters induce inflammation and epidermal hyperplasia in the mouse skin model system (94).

However, in addition to the epigenetic effects of tumor promoters it has also been found that they have an effect on the genetic material of cells. Thus it has been shown that tumor promoters cause gene amplification (95), mitotic aneuploidy in yeast (96), enhancement of irreversible anchorage-independent growth in mouse epidermal cell lines (97), synergistic interactions with viruses in enhancing cell transformation (98), and sister chromatid exchange (99). These genetic events caused by the tumor promoters may be responsible for the irreversible portion of promotion. Furthermore, it has been shown that tumor promoters enhance oncogene-induced transformation of C3H10T1/2 mouse cells (100).

Progressors are carcinogens, tumor promoters or hormones which act on tumor cells to convert them into malignant cells. Like promoters, progressors can cause both epigenetic and genetic alteration in the tumor cells. Progression is often characterized by the occurrence of extensive heterogeneity in the malignant cell population.

Differentiation

The development of malignancy involves genetic changes that uncouple the normal balance between multiplication (growth) and differentiation. There are several ways to uncouple the normal controls for growth and differentiation and several ways for the cells to become malignant.

Evidence obtained with various types of tumors including myeloid leukemias (101) and teratocarcinomas (102) suggests that malignant cells have not lost the genes that control normal growth and differentiation. Phenotypic reversion of malignancy in leukemic cells was obtained by induction of the normal sequence of cell differentiation by the normal differentiation factor (101). In this reversion of the malignant phenotype, halting cell multiplication in mature cells by inducing differentiation by-passes the genetic changes that produced the malignant phenotype. This by-passing of genetic defects is presumably also the mechanism for the reversion of malignancy by inducing differentiation in other types of tumors such as teratocarcinomas, neuroblastomas and erythroleukemias.

An example of tumors which are thought to arise by epigenetic mechanisms is the spontaneous and experimentally-induced teratocarcinomas in mice (102). The interpretation of these results is that potentially reversible, epigenetic changes which occur during the development of these tumors are analogous to changes which occur during normal differentiation.

Similarly, results on the carcinogen induced morphological transformation of early passage Chinese hamster cells (36) and on the radiation-induced transformation of C3H10T 1/2 cells (103) suggested that malignant conversion of these cells may be

due to epigenetic, as well as genetic changes, in order to explain the abnormally high frequencies of transformation.

Hypomethylation

Another type of epigenetic mechanism that could result in the alteration of an oncogene function is hypomethylation. It has been found that several carcinogens, including some for which there is no evidence of covalent interaction with DNA, induce hypomethylation (5, 104–106). Decreased levels of DNA methylation at the 5-position of cytosine (4, 5) induce activity of several genes. Moreover, in some primary human tumors the Ha-*ras* and Ki-*ras* oncogenes were hypomethylated compared to the normal tissues adjacent to the tumor (107). These results suggest that activation of oncogenes can be induced by chemical carcinogens not only by direct DNA damage, but also as the result of stable DNA hypomethylation. It is of interest to note that the 5-methylcytidine analogue, 5-azacytidine, which is incorporated into DNA but cannot be methylated, activates various genes (for a review see ref. 4).

CONCLUDING REMARKS

There is strong evidence to suggest that cancer is a genetic disease at the cellular level. Although environmental factors are of predominant importance in the cause of human cancers, it has been well established that there is also an inherited component. The combination of environmental factors together with inheritance result in the development of cancer.

Advances such as the isolation and characterization of cellular oncogenes, the development of a variety of efficient gene transfer methods, the great sensitivity of nucleic acid hybridization techniques and the production of monoclonal antibodies opened up new areas of cancer research and helped to get a better insight into the causes and mechanisms of carcinogenesis. Oncogene research in particular has accelerated at an explosive rate during the past decade and continues to do so because of its importance in the understanding of human cancer.

The model described in this paper proposes that all forms of cancer are due to a mixture of heritable changes in the cell genome occurring throughout the development of cancer cells and epigenetic changes occurring at the stages of tumor promotion and progression. Conceivably, both genetic and epigenetic mechanisms are associated with the initiation and maintenance of the malignant state, but little is known about the epigenetic contribution to these states. This model is supported by *in vitro* cell transformation experiments and is also consistent with the findings that some oncogenes at least are not tissue specific. This model represents the first effort to incorporate all genes which may contribute to cancer into a unified framework. It is my hope that this will stimulate further thought and experimentation in this area of research.

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REFERENCES

1. Doll, R. and Peto, R. (1981). The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **66**:1193–1308.
2. Varmus, H. (1981). The molecular genetics of cellular oncogenes. *Ann. Rev. Genetics* **18**:553–612.
3. Marshall, C. J. (1985). Human oncogenes. In: *RNA Tumor Viruses. Molecular Biology of Tumor Viruses, 2nd edn.* (R. A. Weiss, N. M. Teich, H. E. Varmus and J. Cofin, Eds.), Cold Spring Harbor Lab., New York. Vol. 22, pp. 486–558.
4. Doerfler, W. (1983). DNA methylation and gene activity. *Ann. Rev. Biochem.* **52**:93–124.
5. Nyce, J., Weinhouse, S. and Magee, P. N. (1983). 5-Methylcytosine depletion during tumour development: An extension of the miscoding concept. *Br. J. Cancer* **48**:463–475.
6. Boveri, T. (1929). *On the Problem of the Origin of Malignant Tumors.* Baltimore: Williams and Wilkins (translated by M. O. Boveri).
7. Foulds, L. (1954). The experimental study of tumor progression. A review. *Cancer Res.* **14**:327–339.
8. Huebner, R. M. and Todaro, G. J. (1969). Oncogenes of RNA tumor viruses as determinants of cancer. *Proc. Natl. Acad. Sci. USA* **64**:1087–1094.
9. Temin, H. M. (1971). The protovirus hypothesis: speculations on the significance of RNA-directed DNA synthesis for normal development and for carcinogenesis. *J. Natl. Cancer Inst.* **46**:3–7.
10. Comings, D. E. (1973). A general theory of carcinogenesis. *Proc. Natl. Acad. Sci. USA* **70**:2324–2328.
11. Cairns, J. (1981). The origins of human cancers. *Nature* **289**:353–357.
12. Weinstein, I. B., Gattoni-Celli, S., Kirschmeier, P., Lambert, M., Hsiao, W., Backer, J. and Jeffrey, A. (1984). Multistage carcinogenesis involves multiple genes and multiple mechanisms. In: *Cancer Cells* (A. J. Levine, G. F. Vande Woude, W. C. Topp and J. D. Watson, Eds.), Cold Spring Harbor Laboratory, New York, USA. Vol. 1, pp. 229–237.
13. Spandidos, D. A. and Wilkie, N. M. (1984). Expression of exogenous DNA in mammalian cells. In: *In Vitro Transcription and Translation—A Practical Approach* (B. D. Hames and S. J. Higgins, Eds.), IRL Press, Oxford, UK, pp. 1–48.
14. Wyke, J. A. and Weiss, R. A. (1984). The contribution of tumor viruses to human and experimental oncology. *Cancer Surveys* **3**:1–24.
15. Knudson, A. G. (1985). Hereditary cancer oncogenes, and antioncogenes. *Cancer Res.* **45**:1437–1443.
16. Spandidos, D. A. (1985). Mechanism of carcinogenesis: the role of oncogenes, transcriptional enhancers and growth factors. *Anticancer Res.* **5**:485–498.
17. Spandidos, D. A. and Siminovitch, L. (1985). Transfer of anchorage independence by isolated metaphase chromosomes in hamster cells. *Cell* **12**:675–682.
18. Spandidos, D. A. and Siminovitch, L. (1978). Transfer of the marker for the morphologically transformed phenotype by isolated metaphase chromosomes in hamster cells. *Nature* **271**:259–261.
19. Shih, C., Shilo, B.-Z., Goldfarb, M. P., Dannenberg, A. and Weinberg, R. A. (1971). Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc. Natl. Acad. Sci. USA* **76**:5714–5718.
20. Sandos, E., Sukumar, S., Martin-Zanca, D., Zarbl, H. and Barbacid, M. (1985). Transforming *ras* genes. In: *Viruses and Cancer* (P. W. J. Rigby and N. M. Wilkie, Eds.), Cambridge University Press, pp. 291–313.
21. Ponder, B. A. J. (1980). Genetics and cancer. *Biochem. Biophys. Acta* **605**:369–410.

22. Lehman, A. R. (1982). Xeroderma pigmentosum, Cockayne syndrome and ataxia-telangiectasia: Disorders relating DNA repair to carcinogenesis. *Cancer Surveys* **1**:93–118.
23. Meuth, M., L'Heureux-Huard, N. and Trudel, M. (1979). Characterization of a mutator gene in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. USA* **76**:6505–6509.
24. Thorgeirsson, S. S. and Nebert, D. W. (1977). The Ah locus and the metabolism of chemical carcinogens and other foreign compounds. *Adv. Cancer Res.* **25**:149–193.
25. Sodroski, J., Rosen, C., Goh, W. C. and Haseltine, W. (1985). A transcriptional activator protein encoded by the x-lor region of the human T-cell leukemia virus. *Science* **228**:1430–1434.
26. Miller, R. W. (1970). Neoplasia and Down's syndrome. *Ann. N.Y. Acad. Sci.* **171**:637–644.
27. Benedict, W. F., Murphree, A. L., Banerjee, A., Spina, C. A., Sparkes, M. C. and Sparkes, R. S. (1983). Patient with 13 chromosome deletion: Evidence that the retinoblastoma gene is a recessive cancer gene. *Science* **219**:973–975.
28. Cavenee, W. K., Doyja, T. P., Phillips, R. A., Benedict, W. F., Godbout, R., Gallie, B. L., Murphee, A. L., Strong, L. C. and White, R. L. (1983). Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* **305**:779–784.
29. Murphee, A. L. and Benedict, W. F. (1984). Retinoblastoma: Clues to human oncogenes. *Science* **223**:1028–1033.
30. Koufos, A., Hansen, M. F., Lampkin, B. C., Workman, M. L., Copeland, N. G., Jenkins, N. A. and Cavanee, W. K. (1984). Loss of alleles at loci on human chromosome 11 during genesis of Wilm's tumour. *Nature* **309**:170–172.
31. Cowell, J. K. (1984). Tracking the cancer genes in paediatric predisposition syndromes: opportunity for prenatal diagnosis. *Cancer Surveys* **3**:573–601.
32. Kovelovich, L. (1982). Adenomatosis of the colon and rectum: relevance to inheritance and susceptibility mechanisms in human cancer. *Cancer Surveys* **1**:71–91.
33. Wang, J. L. and Hsu, Y.-M. (1986). Negative regulators of cell growth. *Trends in Biochem. Sci.* **11**:24–26.
34. Land, H., Parada, L. F. and Weinburg, R. A. (1983). Tumorigenic conversion of primary embryo fibroblasts requires at least two co-operating oncogenes. *Nature* **304**:596–602.
35. Scherer, E. (1984). Neoplastic progression in experimental hepatocarcinogenesis. *Biochem. Biophys. Acta* **738**:219–236.
36. Spandidos, D. A. and Siminovitch, L. (1978). The relationship between transformation and somatic mutation in human and Chinese hamster cells. *Cell* **13**:651–662.
37. Farber, E. (1984). Cellular biochemistry of the stepwise development of cancer with chemicals. *Cancer Res.* **44**:5463–5474.
38. Armitage, P. and Doll, R. (1957). A two stage theory of carcinogenesis in relation to the age distribution of human cancer. *Br. J. Cancer* **11**:161–169.
39. Ames, B. N. (1979). Identifying environmental chemicals causing mutations and cancer. *Science* **204**:587–593.
40. Bouck, N. and I Mayorca, G. (1976). Somatic mutation as the basis for malignant transformation of BHK cells by chemical carcinogens. *Nature* **264**:722–727.
41. Yunis, J. J. (1983). The chromosomal basis of human neoplasia. *Science* **221**:227–236.
42. Knudson, A. G. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA* **68**:820–823.
43. Setlow, R. B. (1985). Repair deficient human disorders and cancer. *Nature* **271**:713–717.
44. McKusick, V. A. (1975). *Mendelian Inheritance in Man. 4th edn.* Baltimore: Johns Hopkins University Press.
45. Mulvihill, J. J. (1977). Genetic repertory of human neoplasia. In: *Genetics of Human Cancer.* (J. J. Mulvihill, R. V. Miller and J. J. F. Fraumoni, Eds.), Raven Press, New York, pp. 241–306.
46. Anderson, D. E. (1978). An inherited form of large bowel cancer. Muir's syndrome. *Cancer* **45**:1103–1107.
47. Graham, F. L. and Van der Eb, A. J. (1983). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**:456–461.
48. Sukumar, S., Notario, V., Martin-Zanca, D. and Barbacid, M. (1983). Induction of mammary carcinomas in rats by nitroso-methyl-urea involves the malignant activation of the Ha-*ras*-1 locus by single point mutations. *Nature* **306**:658–661.
49. Balmain, A., Ramsden, M., Bowden, G. T. and Smith, J. (1984). Activation of the mouse cellular Harvey-*ras* gene in chemically induced benign skin papillomas. *Nature* **307**:658–660.
50. Guerrero, I., Corzada, P., Mayer, A. and Pellicer, A. (1984). Molecular approach to leukemogenesis: mouse lymphomas contain an activated c-*ras* oncogene. *Proc. Natl. Acad. Sci. USA* **81**:202–205.
51. Spandidos, D. A. and Kerr, I. B. (1984). Elevated expression of the human *ras* oncogene family in premalignant and malignant tumours of the colorectum. *Br. J. Cancer* **49**:681–688.

52. Slamon, D. J., Dekernion, J. B., Verma, I. M. and Cline, M. J. (1984). Expression of cellular oncogenes in human malignancies. *Science* **224**:256–262.
53. Spandidos, D. A., Lamothe, A. and Field, J. N. (1985). Multiple transcriptional activation of cellular oncogenes in human head and neck solid tumours. *Anticancer Res.* **5**:221–224.
54. Yokota, J., Tsunetsugu-Yokota, Y., Battifora, H., Le Fevre, C. and Cline, M. J. (1986). Alterations of *myc*, *myb*, and ras^{Ha} proto-oncogenes in cancers are frequent and show clinical correlation. *Science* **231**:261–265.
55. Heppner, G. H. (1984). Tumor heterogeneity. *Cancer Res.* **44**:2259–2262.
56. Westerveld, A., Hueijmakers, J. H. J., Van Duin, M., De Wit, J., Odijk, O., Pastink, A., Wood, R. D. and Bootsma, D. (1984). Molecular cloning of a human DNA repair gene. *Nature* **310**:425–429.
57. Spandidos, D. A. and Wilkie, N. M. (1984). Malignant transformation of early passage rodent cells by a single mutated human oncogene. *Nature* **310**:469–475.
58. Williams, A. R. W., Piris, J., Spandidos, D. A. and Wyllie, A. H. (1985). Immunohistochemical detection of the *ras* oncogene p21 product in an experimental tumour and in human colorectal neoplasms. *Br. J. Cancer* **52**:687–693.
59. Pragnell, I. B., Spandidos, D. A. and Wilkie, N. M. (1985). Consequences of altered oncogene expression in rodent cells. *Proc. Roy. Soc. London UK* **B226**:107–119.
60. Chang, E. H., Furth, M. E., Scolnick, E. M. and Lowy, D. R. (1982). Tumorigenic transformation of mammalian cells induced by a normal gene homologous to the oncogene of Harvey murine sarcoma virus. *Nature* **297**:479–483.
61. Vousden, K. M. and Marshall, C. J. (1984). Three different activated *ras* genes in mouse tumours: Evidence for oncogene activation during progression of a mouse lymphoma. *EMBO J.* **3**:913–917.
62. Albino, A. P., Lestange, R., Oliff, A. I., Furth, M. E. and Old, L. J. (1983). Transforming *ras* genes from human melanona: A manifestation of tumor heterogeneity? *Nature* **308**:69–72.
63. Thorgeirsson, U. P., Turpeenniemi-Hujanen, T., Williams, J. E., Westin, E. H., Heilman, C. A., Talmadge, J. E. and Liotta, L. A. (1985). NIH/3T3 cells transfected with human tumor DNA containing activated *ras* oncogenes express the metastatic phenotype in nude mice. *Mol. Cell. Biol.* **5**:259–262.
64. Mougnieau, E., Lemieux, L., Rassoulzadegan, M. and Couzin, F. (1984). Biological activities of v-myc and rearranged c-myc oncogenes in rat fibroblast cells in culture. *Proc. Natl. Acad. Sci. USA* **81**:5758–5762.
65. Keath, E. J., Caimi, P. G. and Cole, M. D. (1984). Fibroblast cell lines expressing activated c-myc oncogenes are tumorigenic in nude mice and syngeneric animals. *Cell* **39**:339–348.
66. Little, C. D., Lau, M. M., Carney, D. N., Gazdar, A. F. and Minna, J. D. (1983). Amplification and expression of the c-myc oncogene in human lung cancer lines. *Nature* **306**:194–196.
67. Besmer, P. (1984). Acute transforming feline retroviruses. *Curr. Topics Microb. Imm.* **107**:1–28.
68. Neil, J. C., Hughes, D., McFarlane, R., Wilkie, N. M., Onions, D. E., Lees, G. and Jarrett, O. (1984). Transduction and rearrangement of the myc gene by feline leukemia virus in naturally occurring T-cell leukemias. *Nature* **308**:814–820.
69. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. and Astrin, S. (1981). ALV induced tumours have common proviral integration sites and synthesise discrete new mRNAs; oncogenes by promoter insertion. *Cell* **23**:323–334.
70. Klein, G. (1981). The role of gene dosage and genetic transpositions in carcinogenesis. *Nature* **294**:313–318.
71. Kirsch, I. R., Morton, C. C., Nakahara, K. and Leder, P. (1982). Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. *Science* **216**:301–303.
72. Battey, J., Moulding, G., Taub, R., Murphy, W., Stewart, T., Patter, G., Lenoir, G. and Leder, P. (1983). The human c-myc oncogene. Structural consequences of translocation into the Ig-H locus in Burkitt lymphoma. *Cell* **34**:779–787.
73. Groffen, J., Stephenson, J. R., Heisterkamp, N., De Klein, A., Bartram, C. R. and Grosveld, G. (1984). Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* **36**:93–99.
74. Hayday, A., Gillies, S. D., Saito, H., Wood, C., Wiman, K., Hayward, W. S. and Tonegawa, S. (1984). Activation of a translocated human c-myc gene by an enhancer in the immunoglobulin heavy-chain locus. *Nature* **307**:334–340.
75. Bernard, O., Cory, S., Gerondakis, S., Webb, E. and Adams, J. (1983). Sequence of the murine and human cellular myc oncogenes and two modes of myc transcription resulting from a chromosome translocation in B lymphoid tumours. *EMBO J.* **2**:2375–2383.
76. Erikson, J., Ar-Rushdi, A., Orwinga, J. L., Nowell, P. C. and Croce, C. M. (1983). Transcriptional activation of the translocated c-myc oncogene in Burkitt lymphoma. *Proc. Natl. Acad. Sci. USA* **80**:820–824.

77. Davis, M., Malcolm, S. and Rabbitts, T. H. (1984). Chromosome translocation can occur on either side of the c-myc oncogene in Burkitt lymphoma cells. *Nature* **308**:286–288.
78. Rabbitts, P. H., Forster, A., Stinson, M. A. and Rabbitts, T. H. (1985). Translocation of exon 1 from the c-myc gene results in prolonged c-myc mRNA stability. *EMBO J.* **4**:3727–3733.
79. Eick, D., Piechazyk, M., Henglein, B., Blanchard, J. M., Traub, B., Kofler, E., Wiest, S., Lenoir, G. M. and Bornkamm, G. W. (1985). Aberrant c-myc RNAs of Burkitt's lymphoma cells have longer half-lives. *EMBO J.* **4**:3717–3725.
80. Shtivelman, E., Lifshitz, B., Gale, R. P. and Ganaani, E. (1985). A fused transcript of the abl and bcr genes in chronic myelogenous leukemia. *Nature* **315**:550–554.
81. Reddy, E. P., Reynolds, R. K., Santos, E. and Barbacid, M. (1982). A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* **300**:149–152.
82. Tabin, C. J., Bradley, S., Bargman, C., Weinberg, R., Papageorge, A., Scolnick, E., Dhar, R., Lowr, D. and Chang, E. (1982). Mechanism of activation of human oncogene. *Nature* **300**:143–149.
83. Taparowsky, E., Suard, Y., Fasano, O., Simizu, K., Goldfarb, M. and Wigler, M. (1982). Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature* **300**:762–765.
84. Yuasa, Y., Srivastava, S. K., Dunn, C. Y., Rhim, J. S., Reddy, E. P. and Aaronson, S. A. (1983). Acquisition of transforming properties by alternative point mutations within c-bas/has human proto-oncogene. *Nature* **303**:775–779.
85. Bos, J. L., Toksoz, D., Marshall, C. J., Verlaan-De Vries, M., Veeneman, G. H., Van Der Eb, A. J., Van Boom, J. H., Janssen, J. W. G. and Steenvoorden, A. C. M. (1985). Amino-acid substitutions at codon 13 of the N-ras oncogene in human acute myeloid leukaemia. *Nature* **315**:726–730.
86. Seeburg, P. H., Colby, W. W., Hayflick, J. S., Capon, D. J., Goeddel, D. V. and Levinson, A. D. (1984). Biological properties of human c-Ha-rasl genes mutated at codon 12. *Nature* **312**:71–75.
87. Fasano, O., Aldrich, T., Tamanoi, F., Taparowdky, E., Furth, M. and Wigler, M. (1984). Analysis of the transforming potential of the human H-ras gene by random mutagenesis. *Proc. Natl. Acad. Sci. USA* **81**:4008–4012.
88. Alitalo, K. (1985). Amplification of cellular oncogenes in cancer cells. *Trends in Biochemical Sciences* **10**:194–197.
89. Aoseoki, J. G., Rosen, C. A. and Haseltine, W. A. (1984). Trans-acting transcriptional activation of the long terminal repeat of human T lymphotripic viruses in infected cells. *Science* **225**:381–385.
90. Gaynor, R. B., Hillman, D. and Berk, A. J. (1984). Adenovirus early region 1A protein activates transcription of a nonviral gene introduced into mammalian cells by infection or transfection. *Proc. Natl. Acad. Sci. USA* **81**:1193–1197.
91. Borrelli, E., Hen, R. and Chambon, P. (1984). Adenovirus-2 E1A products repress enhancer-induced stimulation of transcription. *Nature* **312**:608–615.
92. Mechler, B. M., McGinnis, W. and Gehring, W. J. (1985). Molecular cloning of lethal (2) giant larvae, a recessive oncogene of Drosophila melanogaster. *EMBO J.* **4**:1551–1557.
93. Weinstein, I. B. (1981). Current concepts and controversies in chemical carcinogenesis. *J. Supp. St. Cell Biochem.* **17**:99–120.
94. Slaga, T. J. (1983). Cellular and molecular mechanisms of tumour promotion. *Cancer Surveys* **2**:595–612.
95. Varshavsky, A. (1981). Phorbol ester dramatically increases incidence of methorexate-resistant mouse cells: Possible mechanisms and relevance to tumor promotion. *Cell* **25**:561–571.
96. Parry, J. M., Parry, E. M. and Barrett, J. C. (1981). Tumor promoters induce mitotic aneuploidy in yeast. *Nature* **294**:263–265.
97. Colburn, N. H., Former, B. F., Nelson, K. A. and Yaspa, S. H. (1979). Tumor promoter induces anchorage independent irreversibly. *Nature* **281**:589–591.
98. Fischer, P. B. and Weinstein, I. B. (1979). Chemical viral interactions and multistep aspects of cell transformation. In: *Molecular and Cellular Aspects of Carcinogen Screening Tests* (R. Montesano, H. Bartsch and L. Tomates, Eds.), IARC Scientific Publications, pp. 113–131.
99. Kinsella, A. R. and Radman, M. (1978). Tumor promoter induces sister chromatid exchanges: relevance to mechanisms of carcinogenesis. *Proc. Natl. Acad. Sci. USA* **75**:6149–6153.
100. Hsiao, W.-L. W., Cattoni-Celli, S. and Weinstein, I. B. (1984). Effects of 5-azacytidine on the progressive nature of cell transformation. *Science* **226**:552–555.
101. Sachs, L. (1978). Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia. *Nature* **274**:535–539.
102. Mintz, B. and Illmensee, K. (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA* **77**:3585–3589.
103. Kennedy, A. R., Cairns, J. and Little, J. B. (1984). Timing of the steps in transformation of C3H 10T1/2

cells by X-irradiation. *Nature* **307**:85–86.
104. Boehm, T. L. J. and Drahousky, D. (1983). Alteration of enzymatic methylation of DNA cytosines by chemical carcinogens: a mechanism involved in the initiation of carcinogenesis. *J. Nat. Cancer Inst.* **71**:429–433.
105. Wilson, V. L. and Jones, P. A. (1983). Inhibition of DNA methylation by chemical carcinogenesis *in vitro*. *Cell* **32**:239–246.
106. Hoffman, R. M. (1984). Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. *Biochem. Biophys. Acta* **738**:49–87.
107. Feinberg, A. P. and Vogelstein, M. (1983). Hypomethylation of ras oncogenes in primary human cancer. *Biochem. Biophys. Res. Comm.* **111**:47–54.