A General Theory of Carcinogenesis

(genes/viruses)

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ABSTRACT A general hypothesis of carcinogenesis is proposed consisting of the following features: (1) It is suggested that all cells possess multiple structural genes (*Tr*) capable of coding for transforming factors which can release the cell from its normal constraints on growth. (2) In adult cells they are suppressed by diploid pairs of regulatory genes and some of the transforming genes are tissue specific. (3) The *Tr* loci are temporarily activated at some stage of embryogenesis and possibly during some stage of the cell cycle in adult cells. (4) Spontaneous tumors, or tumors induced by chemicals or radiation, arise as the result of a double mutation of any set of regulatory genes releasing the suppression of the corresponding *Tr* genes and leading to transformation of the cell. (5) Autosomal dominant hereditary tumors, such as retinoblastoma, are the result of germ-line inheritance of one inactive regulatory gene. Subsequent somatic mutation of the other regulatory gene leads to tumor formation. (6) The Philadelphia chromosome produces inactivation of one regulatory gene by position effect. A somatic mutation of the other leads to chronic myelogenous leukemia. (7) Oncogenic viruses evolved by the extraction of host *Tr* genes with their conversion to viral transforming genes. As a result, in addition to the above mechanisms, tumors may also be produced by the reintroduction of these genes into susceptible host cells.

There are many theories about what causes cancer. Some have suggested that all tumors are caused by viruses, and yet tumors caused by chemicals and radiation and those associated with autosomal dominant genes might occur independently of viruses. Although chromosomal changes are common they are usually random in nature and probably a secondary phenomena, and yet some aberrations, such as the Philadelphia chromosome, are clearly specific for a specific type of cancer. There are intriguing correlations between carcinogens and mutagens, and somatic cell mutations are frequently implicated, but the manner in which they produce malignancy is vague. Finally, there are many correlations between differentiation and malignancy; recently a number of significant observations have been made about the biology of cancer and cell transformation. I feel that many of these observations can be unified into a general hypothesis which may have some relevance to mechanisms of carcinogenesis. A general outline of the hypothesis is presented in the abstract.

Relevant observations

In Viral-Transformed Cells the Transformed State Depends upon the Continued Expression of a Viral Gene. This hypothesis is indicated by studies of temperature-sensitive mutants of polyoma (1) and avian sarcoma (2) viruses. When cells transformed by these viruses are grown at permissive temperatures, part or all of the transformed phenotype is expressed;

when grown at the nonpermissive temperatures, transformation is suppressed. Additional evidence for the above conclusion comes from the observation of Macpherson (3) that hamster cells transformed by Rous sarcoma virus revert to normal when part or all of the viral genome is lost.

For purposes of the present discussion, genes that code for factors that bring about cell transformation (transforming factors) will be called *Tr* genes and the viral gene involved will be called *Tr_v*.

Temperature-Sensitive Transformation Can Also Be Induced by Chemicals. DiMayorca *et al.* (4) briefly exposed a mouse cell line to a potent mutagen and carcinogen (a nitrosamine) and immediately tested for malignant transformation by plating the cells on soft agar. A transformation frequency of 6×10^{-6} was obtained. The transformed phenotype was temperature sensitive. However, in contrast to the temperature-sensitive viral mutants, this time the permissive temperature allowing the expression of the transformed phenotype was 38°, and the transformed state was suppressed at 32°. Since it can be assumed that a simple chemical could not introduce a *Tr* gene into the host it must have already been present, but its function was suppressed. This host gene will be called *Tr_h* and the suppressing locus *i⁺_{Tr}*. It will be assumed that the nitrosamine caused a temperature-sensitive mutation of *i⁺_{Tr}* → *i^{ts}_{Tr}*. At 38° the *i^{ts}_{Tr}* repressor was inactivated, the *Tr_h* locus activated, and the transformed state expressed. At 32° the *i^{ts}_{Tr}* repressor was not inactivated, the *i^{ts}_{Tr}* repressor was active, the *Tr_h* gene inactive, and the transformed phenotype repressed. If similar mutations occurred spontaneously or were produced by radiation, the same interpretation would hold.

The product of the *Tr_h* loci may either act directly as transforming factors, or they may be enzymes that are responsible for synthesizing small molecules that act to release the cell from its usual controls. Some plant tumors, for example, are capable of autonomous growth because they are able to synthesize simple growth factors that normal cells cannot (5).

There Are Many Similarities Between the Expression of Luxury Genes* and Expression of Malignancy. It has frequently been suggested that differentiation and malignancy are regulated by similar cellular control mechanisms (5-7). As pointed out by Ephrussi (8), there are numerous parallels between the expression of luxury genes and the expression of malignancy.

* Luxury genes are defined as those genes which produce proteins that are synthesized by only some cells.

(a) Both behave as recessive factors. The fusion of a cell line expressing a luxury function with one that does not usually results in a hybrid cell that does not express the luxury function (8). The same is true for malignancy (9).

(b) Both may be re-expressed after the loss of chromosomes from the nonmalignant or nonexpressing parent (8).

(c) Both the expression of malignancy (10) and the extinction of luxury gene functions (8, 11, 12) are affected by gene dosage of the relevant genes.

(d) Both the expression of malignancy (13) and luxury gene functions can be abolished by exposure of the cells to BrdU.

The observations on the suppression of synthesis of luxury gene products in somatic cell hybrids is compatible with a model in which many, but not all (12, 14), luxury genes are negatively controlled by unlinked repressor genes i^+ . In the present model, the different Tr_h loci are conceived to be negatively controlled luxury genes.

The Dinucleotide Profile of Tumor Viruses Resembles that of Mammalian DNA. By nearest-neighbor analysis a frequency distribution of 16 possible dinucleotides can be plotted, and such a distribution is characteristic for DNA of different sources. Subak-Sharpe (15) has made the intriguing observation that the pattern for many oncogenic and other small viruses is quite similar to that of mammals. The most likely explanation is that these viruses have developed by the excision of certain genes from mammals or birds. The ideal locus to excise to produce a tumor virus would be one of the Tr_h loci. In simplified terms oncogenic viruses may represent the transition of $Tr_h \rightarrow Tr_v$, plus the necessary accompanying trappings such as polymerases, replicases, and genes for coat proteins, that are necessary for the independent existence of Tr_v .

This immediately leads one to ask, if the Tr_h loci can get into so much mischief, why keep them around? The logical answer is that they have some necessary function during some stage of the cell cycle, or some stage of embryogenesis. They may, for example, be needed for the burst of cell division during cleavage division, or organogenesis. In this regard, they are just like other differentiated or luxury genes which are expressed in some cells but not in others. Cell aging *in vitro* with stage-III cessation of growth could be secondary to a failure in expressing certain Tr genes during the cell cycle.

The Regulatory Loci Must Be Diploid. Before progressing further, a final aspect of the model must be added, namely, since birds and mammals are diploid, the regulatory locus must be i^{+Tr}/i^{+Tr} . Thus, the following situations are proposed. (a) In a normal cell the Tr_h genes (Tr_{h1} , Tr_{h2} ...) are suppressed by i^{+Tr} genes. (b) Spontaneous transformation or transformation by chemicals or x-rays is the result of a double mutation of a pair of i^{+Tr} genes to i^0Tr . (c) Viral transformed cells are the result of the insertion of the Tr_v gene into certain host cells whose i genes do not suppress the Tr_v locus.

Nonviral transformation is presumed to arise through mutation of the i^{+Tr} loci. The average mutation rate per germ-cell locus in mammals is about 10^{-5} – 10^{-6} per locus per generation (16). The spontaneous mutation rate for nondividing somatic cells has been estimated as approximately 10^{-6} – 10^{-7} per cell per locus per year (17, 18). We can estimate that

there are approximately 10^{13} or less cells in each human organ. If i^{+Tr} organ-specific loci were haploid, there would be about 10^7 transformed cells forming per organ per individual per year, an intolerably high figure. Since the locus is diploid, double $i^{+Tr}/i^{+Tr} \rightarrow i^0Tr/i^0Tr$ mutations would occur at a much lower rate of 10^{-14} per year. These figures are only approximations, and immunologic rejection of some transformed cells also plays an important role. Their purpose is to demonstrate that in this hypothesis, spontaneous, chemically induced, and radiation-induced tumors are assumed to be due to double somatic mutations at i^{+Tr} loci, and this makes spontaneous tumors rare enough for survival. This mechanism is consistent with the correlation between mutagenesis and carcinogenesis, with the increasing incidence of cancer with age, with the additiveness of chemical and radiation effects on the production of tumors and cell transformation (19), and with the somatic mutation theories of cancer (17, 20).

Hereditary Tumors. There are a number of tumors, such as retinoblastoma, multiple polyposis of the colon, and neurofibromatosis, that are inherited as an autosomal dominant mutation. Knudson (18) has provided data that suggest hereditary retinoblastomas and other hereditary tumors (21) are the result of a combination of inherited germ-line mutation plus an acquired somatic-cell mutation. In the present model, his dominantly inherited factor would be an i^0Tr gene for a retina-specific Tr_h gene, and his added somatic mutation would be a mutation of the homologous locus resulting in i^0Tr/i^0Tr and derepression of the Tr_h locus with the development of a retinoblastoma. Since the different Tr_h genes may be only partially tissue specific, there might be a higher incidence of tumors in nontarget tissues. This is seen in some cases. For example, there is an increase in the frequency of other primary tumors in patients with retinoblastoma (22). This type of mechanism could also explain the existence of cancer in certain families (23), including those in which the tumors involve different tissues.

In *Drosophila melanogaster* there is a lethal mutation which, when present in homozygous dosage, results in brain tumors (24). In this model both alleles would be germ-line i^0 mutants or i deletions for brain-specific Tr_h loci.

Hybrid Tumors. A different type of hereditary tumor is seen as a result of the production of hybrids between two different species. One of the best-studied examples is the high frequency of melanomas in the platyfish-swordtail hybrid (25, 26). These tumors are due to the release of normal constraints on the growth of platyfish melanocytes. Genetic studies suggest that in the platyfish there is a $2n$ diploid dosage of a regulator gene product responsible for repressing an unlinked structural color gene (sd). When this species is hybridized to the swordtail, which contains neither gene, there is only a $1n$ dose of platyfish regulatory gene product, and the sd gene is partially derepressed and melanocyte growth is more active. In the backcross between the hybrid and the swordtail, fish are produced which have the sd gene but no regulatory gene or other modifier, and here melanocyte growth is unchecked. Melanomas form, spread down over the dorsal fin, invade the body of the fish, eventually account for up to 65% of the body weight, and the fish dies (26). Here only a simple change of names would make this system compatible with the present model. The regulatory loci would represent i^{+Tr-m}/i^{+Tr-m} genes, and the color genes would be Tr_h loci, specific for melanocytes.

Tumors Associated with Deletions. There have been a number of reports of retinoblastoma associated with an interstitial deletion of the long arm of chromosome 13 (27). Here the deletion of a retina-specific $i^{+}_{Tr_h-13}$ locus on chromosome 13 would lead to $-/i^{+}_{Tr_h-13}$. The subsequent step might be a somatic mutation leading to $-/i^{o}_{Tr_h-13}$ with derepression of the Tr_h gene responsible for a retina-specific transforming factor.

The Philadelphia Chromosome. Most patients with chronic myelogenous leukemia have a Philadelphia chromosome (28), which was assumed to be a deletion of about 50% of the long arm of chromosome 22. On this basis Ohno (29) suggested that there is a leukemia-suppressing locus that is deleted in the Philadelphia chromosome and this, combined with a somatic mutation of the homologous locus, leads to the development of a clone of Ph^1 positive cells and eventual chronic myelogenous leukemia. Recently, however, Rowley (30) has shown that instead of being deleted, a portion of the long arm of chromosome 22 is translocated onto the long arm of chromosome 9. Here then is a specific tumor associated with a specific translocation that does not involve the loss of genetic material. Presumably the regulation of some genes has been altered by position effect. This could represent the change of $i^{+}_{Tr_h-22}$ to $i^{o}_{Tr_h-22}$ by position effect with alteration of the other $i^{+}_{Tr_h-22}$ gene by somatic mutation. 10–15% of patients with chronic myelogenous leukemia do not have the Philadelphia chromosome (31–33). These are usually somewhat older patients and they may represent a double-somatic mutation in the $i^{+}_{Tr_h-22}$ locus.

Malignancy Behaves like a Recessive Trait. The studies of Harris and Klein (9) have shown that when a malignant and a nonmalignant cell line are fused, the hybrid is nonmalignant. It can, however, quickly revert to malignant status upon the loss of chromosomes from the nonmalignant parent, and they suggest this is the reason previously reported hybrids of this type appeared to remain malignant. Others (8, 10, 34) have confirmed these observations. This observation is consistent with the presence of a locus on the chromosomes of the nonmalignant parent that is capable of suppressing malignancy. In the present hypothesis, this is assumed to be a i^{+}_{Tr} gene. Fusion of a nonmalignant i^{+}_{Tr}/i^{+}_{Tr} cell with repressed Tr_h genes, with a i^{o}_{Tr}/i^{o}_{Tr} or a $-/-$ malignant cell with activated Tr_h genes results in a nonmalignant $i^{+}_{Tr}/i^{+}_{Tr} -/-$ or $i^{+}_{Tr}/i^{+}_{Tr} i^{o}_{Tr}/i^{o}_{Tr}$ cell with repressed Tr_h genes.

The Fusion of Malignant Cells Can Result in a Nonmalignant Hybrid. Harris and colleagues (8) have also shown that in some cases the fusion of two different malignant cells can result in a nonmalignant hybrid. This would simply represent complementary i^{+}_{Tr} loci.

Chromosomal Aneuploidy. Although chromosomal aneuploidy is extremely common in cancer cells, the observation that many primary tumors are diploid (35) suggests that aneuploidy is a secondary phenomena. I would suggest that through a pleiotrophic effect of the transforming factor, there can be a destabilization of normal chromosome segregation and an increased frequency of aneuploidy. Selection of different unbalanced genomes then leads to acceleration of autonomous growth, either through activation of additional Tr_h genes by deletion of their i^{+}_{Tr} suppressor loci or by effects on other gene loci that affect the regulation of cell growth.

Thus, with the exception of the Ph^1 chromosome, and 13 deletions in retinoblastoma, all chromosome aberrations are visualized as being secondary to the initial event of either activation of Tr_h genes or insertion of Tr_e genes.

It should be emphasized that not all transformed cells are malignant, as judged by the production of tumors after injection into the proper animals. Here the transformation of cells is considered to be the result of Tr_h or Tr_e gene activity, and these cells may or may not be malignant. If they are not malignant, this property may be acquired through chromosomal aneuploidy.

Balance of Malignancy-Suppressing and Enhancing Genes. Studies by Hitotsumachi *et al.* (36) indicate that the transformation of Syrian hamster cells by polyoma virus is associated with the gain of group-5 chromosomes, and the transformed phenotype is suppressed when these chromosomes are lost and certain others gained. They have interpreted these results in terms of a balance of factors carried on specific chromosomes for the expression or suppression of transformation. Others have also noted nonrandom chromosomal changes associated with specific tumors produced by specific viruses or carcinogens (37). Tumors can also be produced by chemical carcinogens in Syrian hamster cells without specific chromosomal changes as detectable by chromosomal banding (DiPaolo, J. A., personal communication).

When nonrandom chromosome changes are observed, they may represent either the deletion of additional i^{+}_{Tr} genes or duplication of Tr_h genes, or they may represent alteration in the dosage of the other genes that have an effect on cell growth.

Cells Vary in Their Ability to be Transformed by Viruses. When tissue-culture cells are infected with oncogenic viruses, not all cells are transformed, and some genetic types of cells are more readily transformed than others (38). The reasons for this are unknown. Within the framework of the present hypothesis polymorphisms among the i^{+}_{Tr} loci may allow some cells to be transformed by viruses while others are not.

Somatic mutation from $i^{+} \rightarrow i^{o}$ among these genes could explain the observation that the incidence of transformation by simian virus 40 (SV40) is increased by prior x-ray irradiation of tissue cells (39). The same holds for the increased frequency of transformation in phase-III fibroblasts (38). This mechanism could also explain the observation that skin fibroblasts from colonic cancer patients belonging to cancer-prone families are more readily transformed than normal fibroblasts (40). If these individuals possessed a germ-line i_{Tr} mutation, they would be more susceptible to the development of malignancies and their fibroblasts could be more readily transformed. It is also consistent with the synergism between chemical carcinogens and the induction of tumors with viruses (41).

Other factors, independent of the expression of Tr_e loci, but affecting the ability of cells to support replication of the viruses (permissiveness) (42), also play an important role in determining which cells can be transformed and which cannot.

Spontaneous reversion of malignancy

Although malignancies *in vivo* may undergo remission as a result of immunological mechanisms, there are numerous examples of other types of reversion (5). Within the framework of the present model there are several potential mecha-

nisms for such spontaneous reversion. These include (a) loss of Tr_e genes, (b) loss of Tr_h genes by chromosome deletion or loss, or by mutation of the Tr_h loci, (c) further unbalancing of the host genome for other growth factors that neutralize the effect of the derepressed Tr_h loci, or (d) differentiation into a cell in which the derepressed transforming factor is no longer effective.

The latter mechanism could explain the intriguing observation that some highly malignant, undifferentiated cells in teratocarcinomas (6, 43) and neuroblastomas (5, 44) can give rise to differentiated cells that are no longer malignant. An alternative explanation of this phenomena would be that $i^{+}Tr_e$ genes may be inactivated by epigenic mechanisms (45) and reactivated upon the differentiation of the host cells.†

Implications

If the transforming factors are normally produced only in embryonic cells, their inactivation by specific antibodies should inhibit tumor growth whether the tumors were spontaneous or induced by chemicals, radiation, or viruses. On the other hand, if transforming factors are also produced some time during the cell cycle in adult cells, or if the absence of suppressing factor causes tumors, this would not be possible. Experimentally, transforming factors might be assayed for by testing the effect of lysates of transformed cells on the growth (46) or DNA synthesis of confluent layers of non-transformed cells. The development of an assay would allow the isolation of the factors involved. The ability of certain fetal serums to stimulate cell growth (47) and the decreased requirement for such factors by transformed cells may be due to the fact that these serum factors are the same or similar to the transforming factors synthesized by some embryonic or neoplastic cells.

One of the features of this model is its proposal that not all tumors are due to omnipresent viral genomes. The oncogene hypothesis (48) also suggests the presence of structural genes in all cells which are normally repressed and can be activated to produce malignancy either spontaneously or by chemicals or radiation. The oncogene hypothesis, however, suggests that those are vertically transmitted viral genes and all malignancy is in one way or another due to C-type viruses. By contrast, the present hypothesis suggests that: (a) The structural genes involved are a normal part of the genome. (b) There are many such genes, some of which are tissue specific, some are not. (c) These genes have a normal function and are normally activated during some stage of embryogenesis and possibly in adult cells. (d) Oncogenic viruses evolved by the excision of one or more of these genes from the host. (e) The products of these genes may normally be present in small amounts in many tissue fluids. (f) Some tumors may occur independently of viruses. The present model also does not require that the host genome have a copy of all the sequences present in the viruses. Only the Tr_h and Tr_e genes need be similar. The virus can bring in additional sequences which would be unique to malignant cells, and assuming

conservation of the Tr_h and Tr_e genes, a given virus could produce similar malignancies in different mammalian species.

The model is consistent with the recessive nature of malignancy, the development of tumors spontaneously or their induction by chemicals or radiation, the parallels between mutagenic and carcinogenic agents, the hereditary nature of certain tumors, the association of some malignancies with specific chromosomal abnormalities, the variation in transformability of different types of fibroblasts, and the induction of tumors by oncogenic viruses.

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† It should be pointed out that a reciprocal model can be constructed in which the malignancy-suppressing loci are structural genes which are turned on in normal cells and produce products that prevent autonomous growth. Space does not allow development of this alternative, which would also be consistent with most of the above observations.

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