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Somatic Mutation and Cell Differentiation in Neoplastic Transformation

E. Huberman and F. R. Collart

Division of Biological and Medical Research
Argonne National Laboratory
9700 South Cass Avenue
Argonne, IL 60439-4833

Cancer cells are marked by uncontrolled replication and blocked differentiation. Consequently, hypotheses about carcinogenesis should, as part of their premise, consider events that affect cell growth and maturation. In the present paper, we suggest that tumor formation may result from continuous expression of growth-facilitating genes that, as a result of somatic mutations, are placed under the control of genes that are expressed during normal cell differentiation.

Human cells produce and respond to growth- and differentiation-inducing factors (Cohen & Carpenter 1975; Metcalf 1985; Sachs & Lotem 1984). Stem cells begin to mature after the interaction of a specific "inducer" of differentiation with its appropriate cellular receptor. Following this interaction, a series of cellular signals are transmitted from the receptor to the genome causing the activation and expression of specific genes, presumably regulatory genes. The products of these early activated genes cause, through positive or negative controls (e.g., through a "trans"-acting process or ligand receptor interaction), sequential expression of genes that code for the different growth and maturation functions, initially causing cell growth (required for self-renewal) and then cell maturation. For maturation, the "inducer" causes the expression of Gene A, which in turn activates Gene B, which in turn activates Gene C. In turn, Gene C activates Gene D and so forth. Furthermore, the products of some of these genes may also represent the various functions that constitute the differentiated state. The product of one or more of the genes that are expressed at a later part of the maturation process may cause the

suppression of the growth facilitating gene(s) (designated as G in Fig. 1), producing terminal differentiation.

Tumor initiation is thought to involve specific irreversible genetic change(s) in a normal stem cell (Huberman & Barr 1986; Pitot et al. 1984). One of these changes may involve a mutational event (e.g., base pair substitution or addition or deletion of a base or a sequence of bases) in a critical gene (e.g., Gene C in Fig. 1) that is part of the normal sequence of genes whose expression produces terminal differentiation. Thus, following the interaction of a cell with the appropriate "inducer" of differentiation, such a mutation may prevent complete maturation, resulting in an immature, continuously growing cell. A similar situation can also occur following a loss of a gene(s) or gene rearrangement in which a growth control gene--for example, a protooncogene (Bishop 1987)--may become part of the sequence of the activated genes that code for the maturation functions (Fig. 2). As in the previous case, initial interaction with the "inducer" will activate the sequence of genes that result in a mature phenotype. However, this sequential gene activation can be interrupted if a growth control gene(s) does not produce the appropriate signal for completion of the differentiation process (Fig. 2).

Fig. 1. Diagram showing a normal cell maturation pathway. The "inducer" of differentiation interacts with its receptor and causes the expression of Gene A, which in turn activates Gene B, which in turn activates Gene C. In turn, Gene C activates Gene D. Gene D inactivates the gene that facilitates growth, Gene G. The resulting situation is expression of Genes A, B, C, and D, all of which code for cell maturation functions, and suppression of the growth facilitating gene, Gene G. The resulting phenotype will be that of a terminally differentiated cell.

Again, this cell will express some early functions of cell maturation and will grow continuously. When cells with the genetic alterations described above are induced to differentiate, the degree of maturation of these cells will obviously depend on the location of the genetic change in the sequence of the genes that code for the maturation markers. Those occurring early in the process will result in poorly differentiated cells whereas those occurring later will produce cells that exhibit a more mature phenotype.

Different types of inducers may cause the same or a similar maturation phenotype in an affected stem cell. Some of these inducers may activate genes that are located beyond the gene that is altered during initiation; consequently, these cells may still be able to mature. Perhaps this is why some inducers of cell differentiation can cause maturation of cultured tumor cells (Huberman & Callaham 1979; Metcalf 1985; Murao et al. 1983; Rovera et al. 1979; Sachs & Lotem 1984). However, this maturation may not be complete because some genes that are part of the cascade of genes activated during cell differentiation were skipped.

The changes induced during tumor initiation in the cascade of genes that control cell differentiation probably

Fig. 2. Another diagram showing a different differentiation sequence in an initiated cell. As in Fig. 1, the inducer activates Genes A and B, as well as the translocated growth-facilitating gene, Gene G. Because Gene G is not capable of activating Gene C, this gene and Gene D are not expressed. Because of inactivation of gene D, there is also no suppression of the growth-facilitating gene G as is described in Fig. 1. The resulting phenotype is that of a partially differentiated, continuously growing cell.

involve gene inactivation. To obtain continuous cell growth after an interaction with the appropriate "inducer," as described before, the genetic alterations occurring during initiation must take place in both alleles of the affected gene (e.g., as a result of a clastogenic event) or in the unaffected allele of a gene that is already heterozygous for the affected locus because of a previous mutation, as in retinoblastoma in children (Knudson 1985; Murphee & Benedict 1984).

The suggestion that inactivation of genes that control cell differentiation may lead to malignant cell transformation is compatible with experiments showing that fusion of tumor cells with normal or nonmalignant cells or introduction of specific chromosomes from normal cells into tumor cells results in the suppression of malignancy (Sager 1986; Stanbridge 1976; Weissman et al. 1987). The chromosomes from the normal or nonmalignant cells presumably contain unaffected genes that have been inactivated in the tumor cells during tumor initiation. This suggestion can also explain why tumor cells usually do not grow in vitro. The culture medium presumably lacks sufficient amounts of the specific "inducer" of differentiation.

The hypothesis described here suggests that natural tumor promotion may result from an interaction of an initiated cell with a continuously produced "inducer" of differentiation; alternatively, promotion may result from an increased production of a usually suppressed "inducer," perhaps due to aging (e.g., during menopause) or other physiological changes. These "inducers" may cause a clonal expansion of the initiated cells through the expression of growth-facilitating genes (Bishop 1987), which as a result of prior gene mutation, gene loss, or gene rearrangement (during tumor initiation) have been placed under the control of genes that regulate normal cell differentiation. This step should result in the production of benign tumors that stop growing when the promotional stimulus is removed. The same premise explains why environmental tumor promoters introduced into the body through air, food, or skin contact, either cause the production of specific "inducers" or are themselves potent "inducers." In recent years, studies with cultured cells have shown that some classes of tumor promoters (e.g., phorbol diesters, teleocidins, or 2,3,7,8-tetrachlorodibenzo-p-dioxin) can act as potent inducers of cell differentiation (Huberman & Barr 1986; Huberman & Callaham 1979; Vanderbark & Niedel 1984).

The next step in carcinogenesis, i.e., tumor progression (Aldaz et al. 1987; Hennings et al. 1983; Klein & Klein 1985), may also entail changes in the arrangement (e.g., gene amplification) or structure of genes that control cell growth (protooncogenes), resulting in increased cell replication (Fig. 3).

To address the issue of gene rearrangement, we studied carcinogen-induced alterations in a gene that codes for an enzyme involved in the regulation of cell growth: inosine 5'-monophosphate dehydrogenase (IMPDH). An increase in the activity of this enzyme is also associated with development of malignancy and tumor growth progression (Weber et al. 1980). To obtain cells with altered IMPDH activity, we used mycophenolic acid (MPA), a specific highly cytotoxic inhibitor of IMPDH, for selection. Treatment of Chinese hamster V79 cells with a carcinogen produced variant cells resistant to MPA. By using a specific anti-IMPDH antiserum and a specific cDNA probe

Fig. 3. Diagrams showing development of tumor cells. This situation is similar to that shown in Fig. 2 except that the growth-facilitating gene, Gene G, is overexpressed because of gene amplification or gene restructuring (e.g., activation of a potent promoter). Genes A and B, but not C and D, are expressed and Gene G is overexpressed. This situation produces tumor cells that exhibit a partially differentiated phenotype with enhanced cell growth.

isolated from a λgt11 expression library, we showed an increase in the amount and activity of IMPDH in the MPA-resistant cells, which resulted from IMPDH gene amplification (Collart & Huberman 1987). The degree of gene amplification in the different cell variants was positively correlated with resistance to MPA and closely paralleled the amounts of cellular enzyme. These results suggest that a carcinogen insult may affect tumor progression by amplifying genes that control cell growth.

Unlike the genes that control cell differentiation, which are usually inactivated or suppressed during initiation, those control genes [perhaps including the IMPDH gene (Collart & Huberman 1987)] that facilitate cell growth during the other steps most likely exhibit a dominant trait, as was shown for a number of viral and transfectable cellular oncogenes (Bishop 1987). It is also expected that during progression, additional changes may cause this altered growth control gene to be exempt from the "inducer" regulating effect, resulting in uncontrolled cell growth that is independent of the tumor-promoting stimulus [e.g., hormone-dependent tumors that lose this dependency with time (Furth 1982)]. In this context, one should assume that tumor cells that grow in culture are selected for independence from the "inducer" effect unless such a factor exists in the serum that usually supplements tissue culture growth medium. However, tumorigenesis may also result from events that encompass those described for both initiation and progression without necessarily requiring a promotion step, namely through genetic changes that cause an enhanced expression of growth control genes that are not dependent on the action of the "inducer."

In brief, I suggest that tumor formation may result from continuous expression of growth facilitating genes that, as a result of irreversible changes during the initiation step, are placed under the control of genes expressed during normal differentiation. Thus, to understand carcinogenesis, we must decipher the processes that lead to the acquisition of a mature phenotype in both normal and tumor cells and characterize the growth dependency of tumor cells to inducers of cell differentiation. Furthermore, the growth of a variety of tumors may be controlled through the use of inducers of maturation that activate genes located beyond the gene that is altered during tumor initiation. Work supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38.

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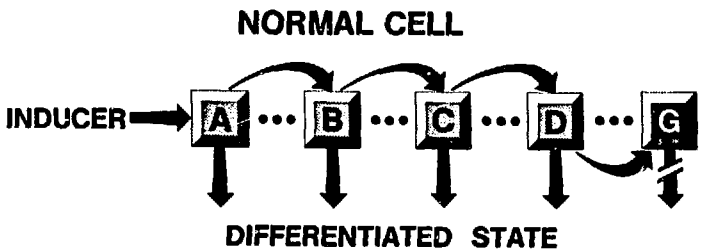

Fig. 1

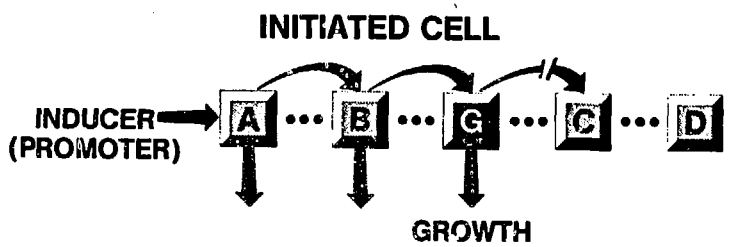

Fig. 2

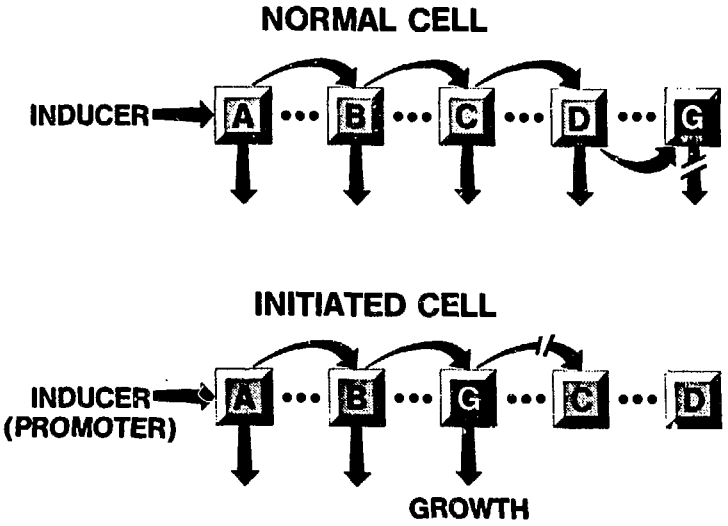
NORMAL CELL
INDUCER
A
B
C
D
G
INITIATED CELL
INDUCER
(PROMOTER)
A
B
G
C
D
GROWTH

Fig. 3