MINIREVIEW

Differentiation Genes: Are They Primary Targets for Human Carcinogenesis?

KEDAR N. PRASAD,*[,1] ALICIA R. HOVLAND,* PIRUZ NAHREINI,* WILLIAM C. COLE,* PETER HOVLAND,* BIPIN KUMAR,* AND K. CHE PRASAD†

Center for Vitamins and Cancer Research, Department of Radiology, School of Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262; and †Department of Pathology, University of California, San Francisco Medical School, San Francisco, California 94131

In spite of extensive research in molecular carcinogenesis, genes that can be considered primary targets in human carcinogenesis remain to be identified. Mutated oncogenes or cellular growth regulatory genes, when incorporated into normal human epithelial cells, failed to immortalize or transform these cells. Therefore, they may be secondary events in human carcinogenesis. Based on some experimental studies we have proposed that downregulation of a differentiation gene may be the primary event in human carcinogenesis. Such a gene could be referred to as a tumor-initiating gene. Downregulation of a differentiation gene can be accomplished by a mutation in the differentiation gene, by activation of differentiation suppressor genes, and by inactivation of tumor suppressor genes. Downregulation of a differentiation gene can lead to immortalization of normal cells. Mutations in cellular proto-oncogenes, growth regulatory genes, and tumor suppressor genes in immortalized cells can lead to transformation. Such genes could be called tumor-promoting genes. This hypothesis can be documented by experiments published on differentiation of neuroblastoma (NB) cells in culture. The fact that terminal differentiation can be induced in NB cells by adenosine 3′,5′-cyclic monophosphate (cAMP) suggests that the differentiation gene in these cells is not mutated, and thus can be activated by an appropriate agent. The fact that cAMP-resistant cells exist in NB cell populations suggests that a differentiation gene is mutated in these cancer cells, or that differentiation regulatory genes have become unresponsive to cAMP. In addition to cAMP, several other differentiating agents have been identified. Our proposed hypothesis of carcinogenesis can also be applied to other human tumors such as melanoma, pheochromocytoma, medulloblastoma, glioma, sarcoma, and colon cancer.

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The human genome is constantly exposed to numerous mutagens and carcinogens. One of the consequences of such exposure is the development of neoplastic diseases. Cancer cells can arise from dividing cells or cells that have the potential to divide. Generically, carcinogenesis is defined as the process during which normal dividing cells accumulate multiple somatic mutations (point mutations and mutations due to chromosomal damage), and then become cancer cells. While most human tumors occur spontaneously, some are age and sex related, and a few are inherited or familial. There is no experimental basis for predicting the latency period for spontaneously occurring human tumors, because many etiologic factors such as genetic history, diet, lifestyle, and environment may contribute to the development of these tumors. In experimental systems the latency period between exposure to carcinogens and appearance of tumors varies depending upon the dose and the type of carcinogen, and the type of experimental model used. In the tissue culture model of normal cells, the latency period is a few weeks, in rodents it is several months, while in humans it ranges from a few years to 30 years or more. The reasons for a long latency period in humans after exposure to carcinogens are unknown. It is certain that the genes responsible for initiating carcinogenesis are not immediately affected by the carcinogens because the surviving cells continue to divide, differentiate, and die in a normal pattern, despite certain gene defects.

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[1] To whom requests for reprints should be addressed at Campus Box C-278, Department of Radiology, University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262. E-mail: Kedar.prasad@uchsc.edu

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The terms "immortalization "and "transformation" are commonly used in the study of *in vitro* carcinogenesis. Occasionally, they are used interchangeably. For the purpose of this review, immortalization refers to the process in which cells lose the capacity to differentiate and then die, and thus continue to divide. Immortalized cells, however, do not produce tumors when injected into athymic mice and therefore, are nontumorigenic. Transformation refers to the process in which immortalized cells acquire the capacity to produce tumors when introduced into athymic mice. Thus, the transition from a normal cell to a cancer cell involves at least two distinct steps: immortalization and transformation. Accordingly, the process of immortalization in normal cells can be called a "tumor initiation phase," whereas the process of transformation of immortalized cells can be referred to as a "tumor promotion phase." Thus, genes that cause immortalization can be considered "tumor initiating genes," and genes that cause immortalized cells to become cancer cells can be considered "tumor promoting genes." In *in vivo* carcinogenesis, the term preneoplastic cells refers to those cells that have an atypical morphology, and that have lost the capacity to differentiate and die in a normal pattern. Such cells can lead to formation of adenomas (the theoretical equivalent of immortalized cells in culture). Cancer cells or neoplastic cells (the equivalent of transformed cells in culture) are those cells that grow like adenoma cells, but that can additionally metastasize to distant organs.

In this review, the term "differentiation genes" refers to those genes that when activated, can induce terminal differentiation in tumor cells, or when suppressed, can cause immortalization. During normal cellular turnover, activation of these differentiation genes is also responsible for inducing differentiation in progenitor cells. The term "oncogenes" refers to proto-oncogenes that when mutated or overexpressed, can increase the risk of cancer. They are also called growth regulatory genes in normal cells.

Several hypotheses have been proposed to explain the biology of carcinogenesis, including a two-stage model (1, 2), chromosomal aberrations (3–5), somatic mutation (6–8), activation of cellular oncogenes due to point mutations and chromosomal re-arrangements (9–13), inactivation of tumor suppressor genes due to point mutations or deletion of chromosome segments (14–17); and downregulation of "differentiation genes" (18).

The purpose of this review is to briefly discuss each carcinogenesis hypothesis and to elucidate our proposed hypothesis that states that downregulation of a differentiation gene may be the primary event that initiates tumorigenesis in humans, and that activation of oncogenes and cellular genes, and inactivation of tumor suppressor genes may be the secondary event. Because of the extensive publications on the proposed topics, only a few selected reviews and references have been included.

Two-Stage Model Hypothesis

This model of carcinogenesis was developed in rodents using pharmacological agents, and it allowed the identification of at least two distinct stages of carcinogenesis, initiation and promotion (1, 2). It was suggested that initiating events occurred at the nuclear DNA level, whereas promoting events occurred at the epigenetic levels (membranes, organelles, and post-translation protein modification). Specific genetic or epigenetic changes, which can be considered causally related to carcinogenesis, were not identified; however, this model has been the most valuable cancer model for public health. It has allowed the identification of many tumor- and antitumor-initiating agents, as well as tumor- and antitumor-promoting agents. These antitumor-initiating and antitumor-promoting agents have been used in several cancer prevention and treatment trials. This model evolved independent of other hypotheses for carcinogenesis that are described below.

Chromosomal Damage Hypothesis

This hypothesis is one of the earliest hypotheses of carcinogenesis proposed by Boveri in 1914 (3), which received support from many studies (4, 5). According to this hypothesis, chromosomal anomalies are the key initial events in carcinogenesis. This is due to the fact that chromosomal damage is often observed in cancer cells, and that all carcinogens (ionizing radiation and chemicals) cause chromosomal abnormalities. This hypothesis has been extensively reviewed (5). It was proposed that chromosomal damage is sufficient and necessary for human carcinogenesis (5). However, it is known that following exposure to carcinogens *in vitro* or *in vivo*, normal human cells continue to proliferate, differentiate, and die in a normal pattern of cellular turnover for some time despite the presence of certain types of chromosomal anomalies. Therefore, chromosomal damage by itself is not always sufficient to induce cancer in normal cells. However, this type of damage can make the genome more sensitive to mutagens, and thus can decrease the latency period during which downregulation of a differentiation gene can occur as a random event.

Somatic Mutation Hypothesis

This hypothesis states that normal dividing cells become cancer cells after accumulation of multiple somatic mutations (6–8); however, the specific gene mutations responsible for immortalization or transformation have not been identified in human cells. Furthermore, not all mutations are considered carcinogenic events; however, they can make the human genome unstable, which leads to an increased accumulation of mutations. When mutations that downregulate differentiation genes occur, normal cells become immortalized. Some mutations can also reduce the latency period.

Viral Infection Hypothesis

Some retroviruses (RNA viruses) cause cancer in rodents, but not in humans (19–23). They include Rous sar-

coma virus (RSV) (19), avian lymphomatosis viruses (24), murine leukemic viruses (21), feline leukemic viruses (25), and murine mammary tumor viruses (19). Some of these viruses are also present in healthy animals (20, 22, 26, 27). Some RNA viruses such as human-T-leukemia virus (28, 29) and hepatitis C virus (30, 31) have been associated with increased risk of T-cell leukemia and hepatic carcinoma, respectively. In contrast to most retroviruses, certain DNA viruses may act as a biological carcinogen. They include simian virus 40 (SV40) (32–35), Epstein-Barr virus (EPV) (36), human papilloma virus (HPV) (37–40), adenovirus (41), and hepatitis B virus (31). The viral genes responsible for immortalization of human cells include the large T-antigen (Lta) gene from the SV40, E6, and E7 from HPV and E1A from the adenovirus. The expression of these genes is necessary for immortalization. For example, the expression of the Lta protein of SV40 virus is for maintaining the cell proliferation of immortalized rat fetal dopamine neurons (42). The inhibition of Lta expression completely blocks cell proliferation (43). This was demonstrated by transplanting immortalized dopamine neurons into the adult rat brain (42). The grafted cells did not divide and did not make Lta within 7 days; however, when these grafted cells were recultured , they started making Lta and cell division was renewed. It is possible that Lta downregulates a differentiation gene. In other cases of viral-induced immortalization, it is also possible that the insertion of a viral gene downregulates a differentiation gene by indirect mechanisms, and thus causes immortalization.

Bacterial and Parasitic Infection Hypothesis

Recently, epidemiological studies have shown that certain bacteria such as *Helicobacter pylori* increase the risk of gastric cancer, which is the most prevalent type of cancer in developing countries (44). In addition, some parasites such as *Opistorchis viverrini* and *Schistosoma haematobium* are considered risk factors for cholangiocarcinoma and urinary bladder cancer (44). A latency period of at least 14 years of chronic infection is needed for the development of these tumors. The removal of these bacteria from the gastrointestinal tract reduces the risk of gastric cancer. However, there are no direct experiments in which human normal cells infected with any of these bacteria are immortalized or transformed. The mechanisms by which *H. pylori* increases the risk of gastric cancer remain unknown. Irritation, inflammation, and induction of increased division rate could be involved as risk factors for carcinogenesis following bacterial infection. Generation of free radicals and release of cytokines during chronic infection may be one of the common intermediary risk factors that increases the risk of cancer in infected organs.

Oncogene Hypothesis

This hypothesis predicts that mutation or overexpression of cellular proto-oncogenes are the primary events that initiate neoplastic changes (9–13). This concept emerged when several viral oncogenes such as v-src, v-ras, and v-myc were isolated from retroviruses and were found to be tumorigenic in rodent cells, suggesting that they act as direct biological carcinogens. Direct transfection experiments with these viral oncogenes in normal rodent cell lines *in vitro* produced transformed cells, confirming that these viral oncogenes act as direct carcinogens in rodents. Over 30 viral oncogene counterparts (cellular proto-oncogenes) from the mammalian genome have been isolated and extensively studied. None of the mutated or overexpressed proto-oncogenes have been able to immortalize or transform human epithelial cells *in vitro*, although some of them such as c-myb can transform rodent hematopoietic cells in culture (45). Thus, mutation in cellular proto-oncogenes cannot be considered an initial event in human carcinogenesis. This is further supported by the fact that only 10% to 50% of specimens of most human tumor tissues have mutations in specific oncogenes (46, 47). The same tumor may have mutations in more than one cellular oncogene, suggesting that they may be secondary events in human carcinogenesis. For example, increased expression of multiple oncogenes such as ras, myc, c-erbB2, and Bcl2 occur in human lung cancer (48). About 30% of adenocarcinomas show mutation in K-ras, and c-erbB2 is overexpressed in up to 25% of non-small-cell lung cancer (NSCLC) cases. From these studies, it not possible to determine which of these gene defects are primary and which are secondary events in human carcinogenesis. Some evidence suggests that mutated cellular proto-oncogenes may act as tumor promoters rather than as tumor initiators. For example, when SV40-induced immortalized human bronchial epithelial cells were transfected with a mutated ras, they became transformed (49). When human fibroblasts immortalized by V-myc were transfected with N-ras oncogene, they became transformed (50).

A recent study has shown successful transformation of human epithelial cells and fibroblasts by transfection with the catalytic subunit of telomerase (hTERT) in combination with SV40 large T antigen gene and H-rasV12 (51). It was suggested that maintenance of telomerase activity may be important in human carcinogenesis. However, others have reported that telomerase-induced immortalized human fibroblasts could not be transformed by ras and human papilloma virus E6 and E7 genes (52). This study suggests that telomerase activity has no direct role in human carcinogenesis. Thus, the role of telomerase activity in human carcinogenesis remains uncertain. It is possible that DNA viral-immortalizing genes or activated telomerase downregulate a differentiation gene that causes immortalization in normal human cells, and that additional gene defects in oncogenes, growth regulatory genes, or tumor suppression genes are needed for transformation.

Tumor Suppressor Gene (Anti-Oncogene) Hypothesis

The tumor suppressor gene concept was derived from the cytological study of retinal cells and retinoblastoma

cells in which one of the alleles of chromosome 13 was missing from the retinal cells, and both were missing from the retinoblastoma cells. This suggested that chromosome 13 must carry a tumor suppressor gene, the loss of which was essential for tumor formation (53). This concept was further supported by somatic cell hybridization experiments in which normal cells were fused with cancer cells, and some of the resultant hybrid clones became nontumorigenic (54). This suggested that chromosomes from the normal cells must contain genes that suppress tumorigenicity of cancer cells.

The first tumor suppressor gene, Rb, was isolated from human retinoblastoma cells by binding it with the LTa of SV40 virus (55). Since it was located on the same chromosome whose loss was necessary for the development of tumor, and since it binds with oncogenic LTa of SV40 virus, it was designated as a tumor suppressor gene. The Rb gene was further found to be present in many normal and tumor tissues. However, in most subsequent experiments, transfection of well-established human cancer cells with wild-type Rb gene inhibited cell growth (56), but it did not convert them to a nontumorigenic state. Wild-type Rb gene also inhibited the growth of normal cells (57); therefore, it is also called a growth regulatory gene. In some tumor cell clones such as Rb-deficient human tumor cells, replacement with the wild-type Rb gene does suppress tumorigenicity *in vitro* and *in vivo* (58).

Another well-known tumor suppressor gene, p53, is also called a growth regulatory gene in human cells (58, 59). On the basis of a high incidence (50%–60%) of mutations in p53 gene in human malignancies, and interaction of wild-type p53 with oncoproteins of tumor viruses, it has been proposed that p53 gene is a common cellular target for human carcinogenesis (60). However, there is no evidence that the insertion of mutated p53 into normal human cells in culture causes immortalization or transformation. Therefore, a mutation in p53 cannot be considered a primary event in human carcinogenesis. Mutations in p53 are present in up to 50% of NSCLC and in 80% of SCLC (48). Activation of either single or multiple oncogenes and inactivation of a single or multiple tumor suppressor genes can occur in other tumors. Similarly, the high expression of wild-type p53 in most human cancer cells has seldom converted them to nontumorigenic cells. In contrast to human cells, the wild-type p53 inhibits E1A plus ras or mutant p53 plus ras-induced transformation of rat embryo fibroblasts (61). Thus, in rodent cells, p53 acts as a tumor suppressor gene—at least against viral oncogene transformation.

Tumor suppressor activity has been reported for several other genes, which include NO3 (62), α-actinin (63), and p16 (64). Genes (wild type) that exhibit tumor suppression activity when transfected into well-established human cancer cells include retinoic acid receptor β (RARβ). This gene codes for a nuclear receptor for retinoic acid, and when it is transfected into human epidermoid lung carcinoma, it causes lose of tumorigenicity potential when tested in athymic mice (65). Other tumor suppressor genes include Normal Epithelial Cell Specific 1 (NES1), a serine protease-like gene (66), Deleted in Colon Cancer (DCC) gene (67), Adenomatous *Polyposis Coli* (APC) gene (68), Maspin, a gene encoding a protein related to the serpine family of protease inhibitors (69), and melanoma associated gene (mda-7) gene (69).

It is interesting to note that in human cells, multiple tumor suppressor genes exist, each of which can be independently regulated, and each of which can influence different cell signaling pathways for growth, differentiation, and transformation. The existence of mutations in multiple tumor suppressor genes in the same tumor suggests that random mutations can affect all genes, including tumor suppressor genes, and that these genes become more susceptible to mutation in a genetically unstable cellular environment. Mutations in tumor suppressor genes can downregulate a differentiation gene or can cause transformation in immortalized cells. Additional mutations in other oncogenes and cellular genes can occur independent of each other without having significant impact on growth regulatory mechanisms.

It has been reported that when human fibroblasts were transfected with a mutated p53 gene, no immortalization occurred; however, when mutated p53-transfected human fibroblasts were treated with a chemical carcinogen, 4-nitroquinoline-1-oxide or x-rays, they became immortalized (70). Furthermore, when papilloma virus-induced immortalized human cervical cells were transfected with mutated ras, they became transformed (71). These studies suggest that mutated p53 and c-ras also may act as a tumor promoter, at least under these experimental conditions.

Telomerase Hypothesis

Recently, telomerase enzyme has generated a lot of interest among cell biologists, developmental biologists, and cancer researchers because of its potential role in proliferation, aging, and carcinogenesis. The human telomerase enzyme consists of at least three components including an RNA (hTERC), a telomerase-associated protein (TEP1), and a telomerase catalytic subunit (hTERT) with sequence similarity to reverse transcriptase enzyme. Most adult normal human somatic cells exhibit little or no telomerase activity; however, several studies have shown that telomerase activity is expressed in germ cells (72), embryonic stem cells (73), fetal liver (74), lymphocytes and hematopoietic progenitor cells (75–77), epithelial cells (78), and endothelial cells (79). It has been proposed that shortening of telomerase after each division eventually leads to senescence (80, 81). Because of the presence of high levels of telomerase (82–85), it has also been proposed that increased telomerase activity is a critical event for continuous cell proliferation of immortalized cells. Others have suggested that both telomerase activity and inactivation of Rb/P16 INK4a are required to immortalize human epithelial cells (86). It has been shown that human breast epithelial cells with stem cell

features are more sensitive to telomerase activation and immortalization by SV40 Lta gene than those with a more differentiated phenotype (87); however, activation of this enzyme does not occur until middle or late passages. This suggests that increased telomerase activity is acquired subsequent to immortalization. Another study has reported that the transfection of human cells with SV40 LTA, mutated Ras, and telomerase genes converted them to cancer cells (51), suggesting a role for telomerase in human carcinogenesis; however, others could not confirm this using a similar experimental approach in which the normal human cells were transfected with papilloma E6, mutated Ras, and telomerase genes (52). A few studies have shown that immortalization and transformation can occur in the absence of telomerase activity (88–90). Others have proposed that telomerase activity is a biomarker of cell proliferation rather than of transformation (91). These studies suggest that the role of telomerase activity in immortalization and/or transformation is controversial. We propose that increased activity of telomerase occurs subsequent to immortalization and/or transformation, and that it pays no direct role in immortalization.

Differentiation Gene Hypothesis

Although the involvement of differentiation in carcinogenesis has been proposed before (18, 92), the specific genes that induce terminal differentiation in normal or transformed cells have not been identified as of yet. In addition, how this differentiation gene is regulated is not well understood. We propose here that the downregulation of a gene, which induces terminal differentiation in normal cells referred to as a differentiation gene, may be an initiating event that causes them to become immortalized, and that mutations (point mutation or overexpression) in cellular oncogenes, growth regulatory genes, and tumor suppressor genes may represent secondary events that convert immortalized cells into transformed cells. This defect in a differentiation gene prevents the cell from undergoing terminal differentiation and subsequent cell death, and consequently, the cells continue to proliferate (hyperplasia), leading to the formation of preneoplastic lesions such as adenomas, which contain cells that are not cancerous. We further propose that the differentiation gene is regulated by at least two sets of genes, "differentiation suppressor genes" and tumor suppressor genes, in an opposite manner. For example, inactivation of a "differentiation suppressor gene" or activation of a tumor suppressor gene downregulates the expression of a differentiation gene. Mutation in a differentiation gene is sufficient to downregulate this gene. These events have been presented in Figure 1. The evidence for the existence of a differentiation suppressor gene is indirect. For example, some genes that were highly expressed in undifferentiated NB cells became undetectable in cAMP-induced terminally differentiated NB cells (93). Some of them could represent "differentiation suppressor genes." Tumor suppressor genes may also regulate the expression of "differentiation genes" (Fig. 1). Indeed, it has been proposed that tumor suppressor genes may regulate cell differentiation in human myeloblastic leukemia cells (94). Specifically, induction of differentiation was followed by a 25- to 30-fold increase in the nuclear levels of wild-type p53 protein, pRb, p130/Rb2, and p21/Cip, a p53-dependent kinase inhibitor. When the expression of each tumor suppressor gene was inhibited by antisense technology, the capacity to differentiate was lost (94). Thus, a differentiation gene may be downregulated by one of the following mechanisms: mutation within the differentiation gene; overexpression of "differentiation suppressor genes;" and inactivation of tumor suppressor genes (Fig. 1).

The fact that terminal differentiation can be induced spontaneously and by elevating cAMP levels in NB cells *in vitro* and *in vivo* (95, 96) suggests that a differentiation gene is downregulated in these cells and that it can be reactivated by the appropriate agents. It is possible that an elevation of cAMP activates a wild-type tumor suppressor gene or inactivates a differentiation suppressor gene. The existence of cAMP-resistant cells suggests that either a differentiation gene is mutated or the regulatory genes have become unresponsive to cAMP. In our preliminary experiments, we have identified some candidate "differentiation suppressor genes" that are downregulated (N-myc, cyclin B_1, and protease nexin 1), and some candidate differentiation genes (c-fos, c-fes, and RAG-1 gene activator) that are upregulated during cAMP-induced terminal differentiation of NB cells (93).

A rational hypothesis must predict certain concepts that can be experimentally tested. Our proposed differentiation gene hypothesis for carcinogenesis predicts the following concepts.

Tumor Cells Can Be Induced to Differentiate Terminally by Appropriate Agents if the Differentiation Gene Is Not Mutated; Conversely, Tumor Cells with the Mutated Differentiation Gene Can Be Resistant to Terminal Differentiation. It has already been demonstrated that an elevation of cAMP in NB cells induces terminal differentiation in most cells in spite of the presence of extensive chromosomal anomalies and mutations (95, 96). This suggests that a differentiation gene was downregulated due to alterations in the regulatory genes rather than to a mutation in a differentiation gene (Fig. 1). The existence of cAMP-resistant NB cells suggests that such cells may have a mutated differentiation gene.

Tumor Cells Can Be Induced to Differentiate, but the Differentiated Phenotype Is Reversible upon the Removal of the Inducing Agent Due to Rapid Hydrolysis of Inducing Signals or to Unresponsiveness of Regulatory Genes. This prediction has been confirmed in non-neural tumors such as glioma cells and sarcoma cells. In non-neural tumors such as rat glioma cells (97) and murine sarcoma cells (98), an elevation of the intracellular level of cAMP induces differentiation; however, removal of cAMP from the culture medium causes reversion to a cancer phenotype (97, 98). These stud-

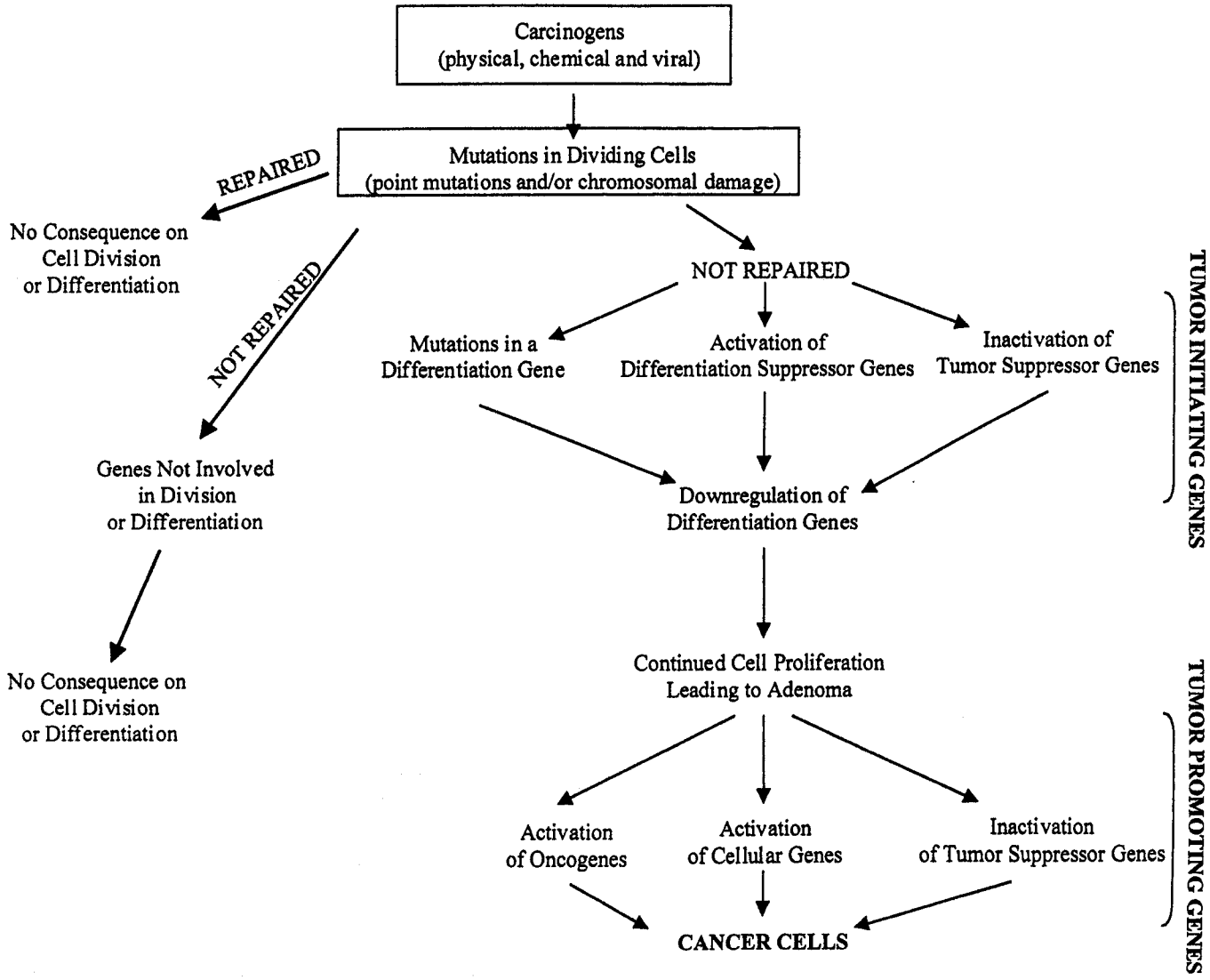

Figure 1. A diagrammatic representation of steps involved in human carcinogenesis following exposure to physical, chemical, and biological carcinogens. Many of the surviving somatic cells may sustain mutagenic changes due to point mutation and/or chromosomal damage. If mutations are repaired or if mutations occurred in genes that are not involved in differentiation or proliferation, carcinogen-exposed cells continue to divide like unexposed cells. If mutation occurred in differentiation genes, tumor suppressor genes, or differentiation suppressor genes, and they are not repaired, downregulation of a differentiation gene can cause immortalization. Immortalized cells can then (due to mutations in growth regulatory genes, cellular oncogenes, or tumor suppressor genes) become cancer cells.

ies suggest that most glioma cells do not have mutations in the differentiation gene, and that elevated cAMP can induce differentiation in these cells by inactivating "differentiation suppressor genes" or activating tumor suppressor genes (Fig. 2). The failure of these tumor cells to maintain a differentiated phenotype upon the removal of chemical stimulus appears to be due to the fact that elevated cAMP is rapidly hydrolyzed (99). Indeed, glioma cells, unlike NB cells, do not elevate the level of cAMP binding that could have protected cAMP from hydrolysis (99, 100). The reversibility of cAMP-induced differentiation in glioma cells thus appears to be due to a subsequent defect in the regulation of cAMP-binding protein (RI). This defect could have occurred at a later stage of tumor development.

Induction of terminal differentiation in well-established cancer cells is possible only when the "differentiation gene" is not mutated, and when the activity of the "differentiation

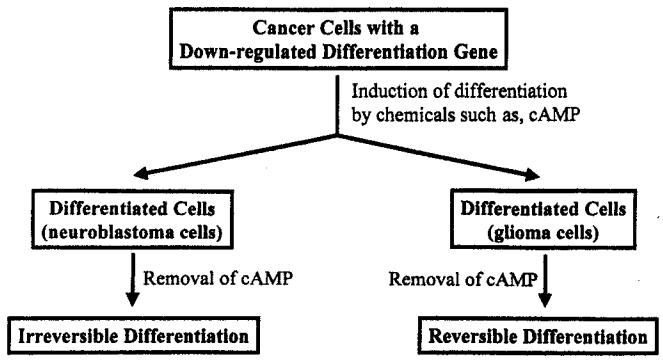

Figure 2. A diagrammatic representation of the response of cancer cells, with downregulation of a differentiation gene, to differentiating agents. Cancer cells could become irreversibly or reversibly differentiated. The reversibility of differentiation upon removal of stimuli suggests the presence of an additional defect in cancer cells subsequent to downregulation of a differentiation gene.

suppressor genes" can be inactivated or the activity of tumor suppressor genes can be activated by experimental agents. NB tumor cells in culture, in which over 95% of cells can be induced to differentiate terminally by experimental agents, provides a unique opportunity to identify a "differentiation gene," "differentiation suppressor genes," and "tumor suppressor genes," and to define their relationship with respect to carcinogenesis.

In addition to NB and glioma cells, our proposed concept to carcinogenesis also applies to other tumors such as melanoma, pheochromocytoma, medulloblastoma, sarcoma, leukemia, and colon cancer. In addition to cAMP, other differentiating agents such as retinoic acid, α-tocopheryl succinate, nerve growth factor, and butyric acid have been identified.

In a recent review on human carcinogenesis, six alterations in cell physiology that collectively define malignant growth have been proposed (101). They include self-sufficiency in growth signal, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion, and metastasis. Extensive alterations of cell signaling pathways and growth regulatory genes are associated with each of the above physiological alterations of cancer cells. They can be used as targets for developing novel anticancer agents. In our view, the six alterations in cell physiology of tumor cells refer to their behavior in the host, and do not refer to the causes of immortalization or transformation. It can be proposed that the first four alterations in the physiology of cancer cells develop subsequent to immortalization, which is induced by downregulation of a differentiation gene; the remaining two alterations in the cell physiology of tumor cells refers to their progression and metastasis in the host. The genes that control metastasis have not been adequately defined.

Rationale for a Long Latency for Cancer Following Exposure to Carcinogens

The existence of a long latency period after exposure to carcinogens in humans can be explained by our proposed hypothesis (Fig. 1). We suggest that initial somatic mutations (gene mutations and mutations due to chromosomal damage) following exposure to carcinogens do not occur in genes that are involved in differentiation; therefore, exposed cells continue to proliferate, differentiate, and die for several years in a pattern that is similar to that found in unexposed cells in spite of genomic instability. It is only when the differentiation gene in a dividing cell is downregulated that the affected cell does not differentiate and thus does not die. It consequently continues to divide (hyperplasia), leading to preneoplastic lesions such as adenomas. Random mutations continue to occur in hyperplastic cells for several years without transformation; however, it is only when mutations occur in the transforming genes (growth regulatory genes, cellular proto-oncogenes, and tumor suppressor genes) of hyperplastic cells, that they become cancerous (transformed). The fact that foci of cancerous cells are found at different locations in polyps exhibiting defects in different transforming genes suggests that mutations in transforming genes occur independent of each other, and that cancerous foci can appear at different times. Mutations are random events, and the probability of them occurring in a specific gene regulating differentiation or transformation is extremely small. Therefore, it takes a long time before such a defect can occur, despite the genetic instability resulting from exposure to carcinogens. Thus, we propose that neither the differentiation gene nor its regulatory genes (differentiation suppressor gene and tumor suppressor genes) are directly affected by carcinogens immediately after exposure. However, exposure to carcinogens can create genetically unstable cells that are more prone to mutagenic changes. When mutations in a differentiation gene or its regulatory genes occur, the cells first become immortal and then, following mutations in transforming genes, they become cancerous. This is supported by the fact that exposure to carcinogens increases the incidence of cancer and decreases the latency period.

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