

Endogenous Carcinogenesis: The Role of Tumor Promotion (43304)

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That environmental factors are predominant in the causation of human cancer is now regarded as a fact. It has been variously estimated (1) that up to 80–90% of neoplasms in humans result from such factors. Diet and smoking probably account for the greatest proportion of these neoplasms, but infectious agents, industrial and occupational exposures, and ultraviolet radiation also play significant roles (2). Yet, with the exception of tobacco abuse as a direct causative factor in the development of bronchogenic and bladder carcinoma, the exact mechanism and proportion of cancers attributable to other agents is not clear. Furthermore, although endogenous hormonal factors play a role in the relationship of parity to breast cancer incidence (3) and possibly to ovarian cancer (4), endogenous factors in the causation of human cancer have only been hinted at. Henderson and his associates (5) have suggested that endogenous estrogens may play a significant role in the development of a number of human cancers. A role of endogenous prolactin in the genesis of mammary cancer in the human has not been proven (6), although this hormone is a significant factor in the genesis of mammary neoplasia in various other species (7). This brief review will consider the contribution of endogenous factors to carcinogenesis both in the human and in experimental animals, with special emphasis on the role of endogenous promoting agents in the development of both spontaneous or fortuitous cancer development, as well as on cancer induced by exogenous agents.

Carcinogenesis by Endogenous Factors

Historically, one of the earliest suggestions that endogenous factors might be important in the development of a variety of cancers both in the human and in experimental animals arose from the observation of the structures of some polycyclic carcinogenic hydrocarbons and naturally occurring bile acids (quoted in

Ref. 8). Although the direction of these theories implied a chemical conversion *in vivo* of naturally occurring metabolites to chemicals similar to carcinogenic polycyclic hydrocarbons—a conversion never substantiated—today it is apparent that both endogenous sex steroids and several of the bile acids produced in the gastrointestinal tract may play significant roles in carcinogenesis in the human (5, 9). High doses of natural estrogens given to animals result in neoplasms of the kidney (10) and Leydig cells (11). Even more potent as chemical carcinogens are a number of synthetic estrogenic compounds, including diethylstilbestrol (12), ethinyl estradiol, and several other similar compounds (13). In addition, synthetic androgens have been identified as potential hepatocarcinogens both in the experimental animal (14) and the human (15). A summary of these agents and the neoplasms induced is given in Table I.

Although other low molecular weight endogenous effectors have not exhibited significant carcinogenic activity, a number of polypeptides exhibit demonstrable carcinogenic effects in a variety of different systems (Table I). Two classic examples of the carcinogenic effects of polypeptide hormones are exhibited by the surgical transplantation of ovarian tissue into the spleen of the castrate mouse and the induction of thyroid neoplasia as a result of the excess chronic production of thyrotropin from a transplanted hormone-producing pituitary neoplasm (16). In the first example, carcinogenesis was presumed to result from the excessively high levels of gonadotropic hormones produced by the pituitary gland in the absence of feedback control by estrogens owing to their metabolism by hepatic tissue receiving the steroid hormones from the splenic ovarian transplants. The continued and excessive stimulation of the end organ by its pituitary trophic hormone, of which the thyrotropin-thyroid model was one of the first, is probably a general effect. Other examples may include the development of acidophilic pituitary neoplasms in old gonadectomized rats (cf., 17), and adrenocortical hyperplasia and adenomas resulting from excessive adrenocorticotropin production, either ectop-

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Table I. Carcinogenic Agents as Components of Endogenous Homeostatic Processes in Mammals^a

Endogenous carcinogens	Target organ
Steroids	
Estrogen (natural)	Kidney, Leydig cells
Estrogens (synthetic)	Liver, mammary and pituitary gland
Androgens (synthetic)	Liver
Bile acids	Colon (?)
Polypeptides	
Prolactin	Mammary gland
Growth hormone	Bone
Thyrotropin	Thyroid
Follicle-stimulating hormone	Ovary, testis (?)
Transforming growth factors, epidermal growth factor	"Transformation" of cultured cells ^b

^a See text for appropriate references.

^b This refers to morphological and histological transformation, which reverts to the "untransformed" state on removal of the polypeptide.

ically from other neoplasms or from functioning pituitary adenomas (18).

In addition to the trophic hormones of the pituitary, a number of growth factors produced endogenously have now been described (19). In at least several instances, while permanent neoplasia has not resulted from such growth factor stimulation of target cells, several such growth factors can induce "morphological transformation" of cells in culture (20). Such cells have many, if not all, of the phenotypic characteristics of malignancy.

Thus, it is clear that a number of endogenously produced hormones and growth factors are carcinogenic in appropriate test systems. The next section of this short review will discuss potential mechanisms for the carcinogenesis of such endogenous factors.

Mechanisms of Carcinogenesis by Endogenous Factors

A consideration of the mechanisms of carcinogenesis includes those involving direct interactions with DNA and those concerned with alterations in the expression, but not necessarily the structure, of the genome. Mechanisms involving the indirect alteration of DNA structure have also been postulated to be important in the carcinogenic action of certain agents, especially ionizing radiation. Although the agents listed in Table I are generally thought to exert their effects on living systems by affecting the expression, and not the structure, of DNA, there is increasing evidence that the metabolism of some low molecular weight endogenous carcinogenic agents may induce alterations in DNA by indirect effects (see below).

The Multistage Nature of Cancer Development

A reconciliation of carcinogenesis by either structural or expressional DNA changes may be accomplished by placing the known information on the mechanism of action of a carcinogenic agent in the context

of the multistage nature of neoplastic development (21). The characteristics of each of the three stages in carcinogenesis are depicted in Table II. While the first stage, initiation, and the final stage, progression, appear to involve structural changes in DNA, promotion is characterized by nonpermanent alterations in the proliferation (DNA synthesis) of initiated cells and their progeny and by reversible alterations in genetic expression. Furthermore, it is likely that the structural genomic changes that initiate cells are qualitatively different from those characteristics of the stage of progression (22). In the former instance (initiation), single base changes (transitions and transversion) and/or small deletions in critical genes, possibly proto-oncogenes (23), may be sufficient to convert a normal cell to an initiated cell. For a cell to be in the stage of progression, major changes in the genome, such as chromosomal translocations, deletions, insertions, and gene amplifications, must be evident.

The presence of one or more initiated cells in most, if not all, mammalian tissues that undergo some degree of cell replication during their existence is undoubtedly a common occurrence (21). At least one round of cell division is required for the formation or "fixation" of an initiated cell (21). A similar requirement is necessary to "fix" a mutation in a cell, and, by analogy, the mechanisms are probably the same. Since initiating agents, such as background radiation, dietary contaminants, air pollutants, and exposure to chemicals in the work place or home environment, pharmaceuticals, and other sources, abound in the environment, the possible sources of "spontaneous" initiated cells are manifold. Such a background population of initiated cells can be a source for preneoplastic lesions that may develop from the chronic action of a promoting agent. Since each preneoplastic lesion in the stage of promotion develops as a clone (24), a very few spontaneously initiated cells can develop into individual multicellular lesions, and with continued cell replication, they are at

Table II. Biological Characteristics of the Stages of Initiation, Promotion, and Progression in Hepatocarcinogenesis^a

Initiation	Promotion	Progression
Irreversible, with constant "stem cell" potential	Reversible	Irreversible. Measurable and/or morphologically discernible alteration in cell genome's structure
Efficacy sensitive to xenobiotic and other chemical factors	Promoted cell population existence dependent on continued administration of the promoting agent	Growth of altered cells sensitive to environmental factors during early phase
Spontaneous (fortuitous) occurrence of initiated cells can be demonstrated	Efficacy sensitive to dietary and hormonal factors	Benign and/or malignant neoplasms characteristically seen
Requires cell division for "fixation"	Dose response exhibits measurable threshold and maximal effect dependent on dose of initiating agent	"Progressor" agents act to advance promoted cells into this stage, but may not be initiating agents
Dose response does not exhibit a readily measurable threshold	Relative effectiveness of promoters depends on their ability with constant exposure to cause an expansion of the progeny of the initiated cell population	Spontaneous (fortuitous) progression can be demonstrated

^a Adapted from Pitot (21).

risk to spawn cells into the stage of progression as malignant neoplasms. Karyotypic abnormalities, which are the herald of the stage of progression, can occur in cell populations that exhibit a higher than normal rate of cell proliferation (Table II), such as those in the stage of promotion, especially when the genome is already altered by mutation as believed to occur in the initiated cell and its progeny. Therefore, both the "genetic" stages of carcinogenesis—initiation and progression—can occur fortuitously, and the intermediate stage of promotion, whether induced exogenously or endogenously, can serve to realize the potential for neoplastic development of such spontaneous processes.

Structural versus Expressional Mechanisms of Endogenous Carcinogenic Factors

As indicated in Table I, a number of chemical species produced in the organism have been shown to be carcinogenic. Without exception these agents are classifiable as promoting agents, based both on a direct demonstration of this capacity and on their known functions in the regulation of genetic expression. In retrospect, this is not surprising, since the actions of these agents on cell replication and gene expression are reversible, thus allowing maximal flexibility in maintaining the homeostasis of the organism. Some of the defined and hypothesized mechanisms of these agents are listed in Table III.

Estrogens and, to a lesser extent, androgens are the principal steroid hormones that are carcinogenic *in vivo* (Table I). At the molecular level, their effects on genetic

expression are mediated through their specific interaction with an endogenous receptor protein produced in the target cell. The steroid-receptor complex interacts with specific sequences in the regulatory regions of a number of genes to increase or decrease the transcription of these genes (25). Presumably, one or more of these genes are important in the overall mechanism of tumor promotion by these agents. In one such gene family, the protein products are components of intercellular gap junctions (26). The inhibition of intercellular communication has been related to the mechanism of tumor promotion (27), although in only a few systems has this alteration been experimentally related to a change in the regulation of the expression of the gap junctional proteins, the connexins, in this process (28, 29). Of the potential endogenous carcinogens possessing a steroid nucleus, only certain bile acids have been shown to inhibit intercellular communication in some *in vitro* tests (30). Neither the synthetic estrogen diethylstilbestrol (31) nor the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (32), an exogenous toxin interacting with an endogenous receptor analogous to the mechanism of steroid hormone action, exhibited inhibition of intercellular communication in cultured cells. Unlike the relative lack of demonstrable effect on intercellular communication by steroid hormones, polypeptide growth factors have been shown to inhibit gap junctional communication in mammalian cell lines (33). Another gene whose altered expression in response to estrogen may be important in the promotional effectiveness of this steroid is the epidermal growth factor

Table III. Mechanisms of Action of Endogenous Carcinogenic Agents^a

Agent	Known mechanisms	Postulated mechanisms
Steroid sex hormones	Interaction with specific cytosolic receptor, the complex directly and specifically altering transcription of specific genes.	Formation of free radicals resulting from metabolism of steroid hormones by monooxygenases. Inhibition of individual cell death (apoptosis) in target organs.
Bile acids		Inhibition of intercellular communication.
Polypeptide hormones	Interaction with specific surface membrane receptor, which in turn alters phosphorylation of key metabolic intermediates and proteins, leading to alterations in the expression of specific genes. Stimulation of cell replication in target end organ.	Inhibition of intercellular communication.

^a See text for further discussion and references.

receptor, whose transcription increases significantly following estrogen administration (34).

A major mechanism by which promoting agents exert their effects is the stimulation of cell replication (Table II; 35). This mechanism is most evident in the trophic effects of specific polypeptide hormones, as well as in both the general and specific effects of polypeptide growth factors that exhibit some degree of specificity for tissues and cells *in vivo* and *in vitro* (19). In fact, it has been argued that the principal effect of all promoting agents is their action to increase the rate of cell replication in the target-initiated cell population. Most promoting agents also stimulate cell replication in normal cells that possess a receptor or other "mechanism" allowing the specific interaction of the promoting agent with the cell type involved. However, in order for promoting agents to act selectively during the natural history of neoplastic development, their effects on initiated cells must be more efficient than in normal cells. That such is the case has been demonstrated by Schulte-Hermann and his colleagues (36), who have demonstrated that the progeny of initiated hepatocytes in the stage of promotion exhibit a greater rate of cell replication under the influence of the promoting agent than do normal hepatocytes. However, this may be somewhat difficult to evaluate, since the rate of replication of the progeny of initiated hepatocytes both *in vivo* (37) and *in vitro* (38) is higher than that of normal hepatocytes. Furthermore, Bursch and his colleagues (39) have presented evidence that certain steroid hormone-promoting agents, notably cyproterone acetate, also

possess the capacity to inhibit individual cell death or apoptosis, as compared with the rate of this process in normal hepatocytes. In the final analysis, the overall increase in the population of the progeny of initiated hepatocytes is dependent on the differential rate of cell replication and apoptosis. Furthermore, although cell replication is a necessary component of tumor promotion, it has yet to be proven that it is sufficient for this stage to be effected.

Although the stage of tumor promotion has classically been considered not to involve alterations in the structure of DNA, a number of studies have demonstrated that certain promoting agents either cause, or their metabolism is accompanied by, the formation of active oxygen radicals (40). Such radicals can cause or stimulate the peroxidation of unsaturated lipids and the development of other free radicals that may affect macromolecular structures, especially DNA (41). Specifically, tetradecanoylphorbol acetate stimulates the formation of active oxygen radicals in the accompanying inflammatory infiltrate seen during the stage of promotion in epidermal carcinogenesis (42), as well as in other cells with which it interacts. The metabolism of many xenobiotic promoting agents is also accompanied by the formation of active oxygen radicals (43). A specific class of promoting agents, those which stimulate the proliferation of intracellular peroxisomes, may, by such a mechanism, increase the production of hydrogen peroxide and, indirectly, of active oxygen radicals, thereby exerting their carcinogenic effects (44). While this hypothesis is extremely attractive and in all

likelihood plays some role in the development of neoplasia, a number of inconsistencies do not support the concept that active oxygen radicals play a significant role in the reversible stage of promotion. First, it is difficult to reconcile an irreversible effect on the genetic material with an operationally reversible stage in neoplastic development. Second, there is very little evidence for the production of significant DNA structural changes resulting from the acute or chronic administration of a variety of promoting agents at doses at which they are effective. The action of several promoting agents, especially the polypeptide hormones and growth factors, is not accompanied, either directly or indirectly, by the formation of active oxygen radicals or other free radicals. Since the inhibition of intercellular communication by at least one promoting agent, tetradecanoylphorbol acetate, is not mediated through free radicals (45), polypeptide hormones and growth factors might not be expected to exert their mechanisms of promotion through oxygen radical formation, since it appears they do affect intercellular communication (33). On the other hand, there is significant evidence that the formation of active oxygen radicals may result in clastogenesis, thus effectively inducing the stage of progression in the development of malignant neoplasia (46).

Conclusions

As seen from the above discussion, ample evidence indicates that endogenous promoting agents occur ubiquitously within mammalian organisms and probably within lower forms of life as well. The mechanisms of promotion by these various endogenous agents appear to differ somewhat among the several known classes (Table III). To date, the most critical characteristics of the stage of promotion, as indicated in Table II, are the expansion of the initiated cell population and the alteration of gene expression in this population under the influence of the promoting agent, both characteristics being operationally reversible. While the critical mechanisms for these effects are as yet unknown, several postulated mechanisms have been promulgated, some of which are depicted in Table III. The interesting phenomenon related to the presence of endogenous promoting agents is the relative lack of their effect in ambient situations that are not influenced by external environmental factors, such as may be seen in certain human populations and rodent strains exhibiting very low incidences of spontaneous cancer. One reason for these differences is the genetic constitution of the host, which may dampen the endogenous promoting capacity, as has been reported in the mouse (47). Environmental agents that inhibit promotion are widespread (48), and the fact that such agents exhibit thresholds or no-effect levels (Table II) suggests that under certain physiologic conditions the thresholds for endogenous

promoting agents may not be exceeded. If such a situation occurs even occasionally, the reversible nature of tumor promotion may cancel the previous effect of the endogenous promoting agent in a manner described experimentally by Boutwell (49).

Whatever factors control endogenous tumor promotion, it is critical that both the experimental investigator and the clinician realize the potential for endogenous tumor promotion and the methods for its inhibition and activation as components of the experiment, the environment, or the therapeutic regimen.

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