Stage of Tumor Progression, Progressor Agents, and Human Risk (43511F)

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That cancer develops from an antecedent process consisting of distinct, definable stages or phases is now considered to be the case in the development of the majority of animal and human neoplasms (1–5). In some of the earliest studies in experimental animals demonstrating the existence of such stages (6–8), two stages, termed initiation and promotion, occurred in that order preceding the development of tumors. The majority of tumors occurring in the system studied, that of mouse epidermal carcinogenesis, were benign papillomas, and carcinomas developed only later. Subsequently, Foulds (9), from studies on experimental mammary adenocarcinoma, argued that the stage following initiation be termed "progression," to represent a continuous spectrum of alterations, presumably irreversible, beginning with the initiated cell.

Later investigations, however, have shown that neither of these two concepts could account for all of the biological characteristics of neoplastic development following initiation. The characteristics of initiation—including its irreversibility, additivity, and lack of a readily measurable threshold dose of the initiating agent—have been generally accepted (1, 3). Furthermore, there is increasing evidence that the process of initiation has many, if not all, of the characteristics usually attributed to mutagenesis (10, 11). Indirect evidence (12, 13) has argued that initiation is characterized by relatively minor structural changes in the genome, such as base transitions, transversions, and small deletions. That the process of initiation can and does occur spontaneously in virtually all animal systems is now well recognized (1, 11). Recent evidence (14) has suggested that, while it is possible that millions of cells may be initiated in an organism, those that develop further in the stage of promotion are dependent upon the specific chemical characteristics of the promoting agent (14). This leads to the concept that cells in the stage of initiation are quite common, with those that develop further in the minority.

Postinitiation Stages of Carcinogenesis

As noted above, the terminology for stages beyond initiation has been somewhat confused until recently. However, with a more complete study of the characteristics of postinitiation stages of carcinogenesis and the development of model systems for the study of these stages (15), a more distinct picture of the process of neoplastic development has evolved. At least two distinct postinitiation stages, promotion and progression, occurring in that sequence, can be defined and characterized (1). The characteristics of these two stages are listed in Table I. The critical distinction between the first postinitiation stage, promotion, and the final postinitiation stage, progression, is their reversibility and irreversibility, respectively. Furthermore, many, if not all, of the cells in the stage of promotion are dependent upon the presence of the promoting agent for their continued existence (17, 18). One mechanism for this dependency, which has been demonstrated in the case of tumor promoters involved in hepatocarcinogenesis (19), is the inhibition by the promoting agent of apoptosis, programmed cell death, in cell populations in the stage of promotion. An interesting and as yet little understood fact is that, after regression of lesions in the stage of promotion on removal of the promoting agent, subsequent readministration stimulates regrowth of the lesions, presumably in many cases from one or a few cells that have escaped apoptosis (20).

The irreversible characteristic of the stage of progression is emphasized by the demonstration in cells in this stage of major genetic abnormalities usually discernible as karyotypic anomalies (21), in contrast to the normal chromosomal structure of cells in the stage of promotion (22, 23). Furthermore, although cells in the stage of progression are not completely dependent for their existence on the continued presence of the promoting agent, it is clear that promoting agents and other environmental factors may enhance the development of cells in this stage (Table I). Progressor agents

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Table I. Some Biological Characteristics of Promotion and Progression in Carcinogenesis[a]

Promotion	Progression
Reversible	Irreversible. Measurable and/or morphologically discernible alteration in cell genome's structure
Promoted cell population dependent on continued administration of the promoting agent	Growth of altered cells sensitive to environmental factors during early phase
Efficacy sensitive to dietary and hormonal factors	Benign and/or malignant neoplasms characteristically seen
Dose response exhibits measurable threshold and maximal effect dependent on the total dose of initiating agent	"Progressor" agents act to advance promoted cells into this stage
Relative effectiveness of promoters depends on their ability with constant administration to cause an expansion of the progeny of the initiated cell population	Spontaneous (fortuitous) progression can be demonstrated

[a] Table adapted from Pitot (16).

that act to cause the conversion of cells in the stage of promotion to the stage of progression are genotoxic, possessing the ubiquitous characteristic of clastogenesis in contrast to the action of initiating agents (21, 24), which induce point mutations and small deletions when initiating a normal cell without significant clastogenesis (11–13, 23). Since it is now generally accepted that most mutagenic agents induce most types of mutation, depending to some extent on the dose (25, 26), one may understand why very low doses of complete carcinogens only initiate cells, whereas much higher doses cause the rapid transition of normal cells to the stage of progression, at times with no clear transitional stage of promotion (27, 28). On the other hand, agents may exist that rarely, if ever, actually initiate cells but exhibit clear progressor activity. Clastogenic agents are most likely to be in this category (21, 24). Finally, a characteristic common to all stages of neoplastic development is their spontaneous occurrence. Many endogenous agents are capable of tumor promotion (16), and thus endogenous or spontaneous promotion is present in virtually all species. Spontaneous (fortuitous) progression may be the result of spontaneous abnormalities in cell division, which occur in relatively rapidly dividing populations of initiated cells during promotion in the presence of the promoting agent (29).

Comparison of the Characteristics of Initiating and Progressor Agents

Initiating agents cause the initiation of cells presumably by a mechanism involving a change in the structure of one or more critical genes. The evidence supporting this statement is as follows.

1. All initiating agents are themselves mutagenic or can be metabolized to a mutagenic intermediate (proximate carcinogen) (1, 10, 11).

2. Initiation exhibits no "measurable threshold" in a dose-response relationship (30, 31), which indicates that even minimal changes (mutations) in gene structure can induce the stage of initiation in a cell.

3. Like the process of mutagenesis, initiation (i) is additive with dose to the point of toxicity (11, 31, 32), (ii) requires "fixation" by one or a few cycles of cell division in the presence of the initiator (1, 33), and (iii) induces an "irreversible," permanent phenotype (a presumed reflection of the altered genotype) in the initiated cell (34).

Although this evidence strongly supports the thesis that initiation is the result of a genetic change, the evidence cited above also indicates the general nature of the initiating event. Since very low doses of initiators are effective, the structural DNA changes occurring in the stage of initiation must be very subtle, probably involving transitions, transversions, small deletions, and the like (1). In support of this statement are the characteristics of the mutated sequences in a number of proto-oncogenes (35) and tumor suppressor genes (36) that have been reported. In a significant number of instances, the base change(s) found at the site of mutation is that predicted by the chemistry of the mutational event (37).

On the basis of our knowledge of the stage of progression, progressor agents should also induce structural alterations in the genome. Since, unlike initiation and promotion, cells in the stage of progression are aneuploid, exhibiting discernible chromosomal abnormalities, it is reasonable to propose that the genotoxic nature of progressor agents is their ability to induce, directly or indirectly, karyotypic structural changes. This characteristic can be more fully appreciated from the tabulation of putative progressor agents (Table II; Refs. 38–49). Table II lists representative examples of biological, physical, and chemical agents, all of which are clastogenic and few, if any, of which have significant initiating activity. However, all of these agents are carcinogenic, although the carcinogenic action of some can be demonstrated only by their action on populations of cells in the stage of promotion. These would include benzoyl peroxide, hydroxyurea, and 2,5,2′,5′-tetrachlorobiphenyl. The techniques used to identify

Table II. Putative Progressor Agents in Carcinogenesis in Humans and Lower Animals

Agent	Initiating activity	Clastogenic activity	Carcinogenic activity	Ref.
Hepatitis B virus	?	+	+	38, 39
Herpes simplex virus	–	+	+	40
Ionizing radiation	±	+	+	41
Arsenic salts	–	+	+	42, 43
Asbestos fibers	?	+	+	44, 45
Benzene	–	+	+	46
Benzoyl peroxide	–	+	±	47
Hydroxyurea	–	+	±	21
1,4-Bis[2-(3,5-dichloropyridyloxy)]-benzene	–	+	+	48
2,5,2′,5′-Tetrachlorobiphenyl	–	+	±	49

putative progressor agents active *in vivo* are only now beginning to be developed, as discussed below.

Identification of Progressor Agents

Although all complete carcinogens by definition possess progressor activity, only relatively recently have model systems been developed in which progression, separate from promotion and initiation, has been demonstrable. Among the early examples of such model systems was that of epidermal carcinogenesis, in which the conversion of papillomas to carcinomas was demonstrated by subsequent administration of specific carcinogens to animals bearing papillomas (50). A similar model system has been demonstrated in rat liver (51), as well as in several earlier studies on rat liver and bladder (52–54).

The theoretical basis for such model systems is the "two-hit" hypothesis first enunciated by Knudson and Moolgavkar (55) and their associates, and later elaborated on by Potter (56). A diagram of a model system for the demonstration of initiation, promotion, and progression is shown in Figure 1. In mouse epidermis, initiation is carried out after weaning with promotion until papillomas develop, followed by administration of a progressor agent (50). In rat liver, the system has involved the administration of an initiating agent either during the first week of life or shortly after weaning. If the latter course is taken, a mitotic stimulus in the form of a 70% partial hepatectomy is utilized immediately prior to the administration of the initiating agent. Administration of a promoting agent starts from the time of weaning in the case of neonatal initiation or immediately after the administration of the initiating agent when initiation is performed after weaning. In the latter case, after 4–6 months of promotion, the progressor agent is administered, usually in several or multiple doses. In the case of neonatal initiation, the progressor agent is administered after the 4- to 6-month period of promotion either as a single dose or multiple doses, with a 70% partial hepatectomy administered within 24–48 hr after the operation (51). The necessity for an externally induced mitotic stimulus to stimulate the conversion from the stage of promotion to that of progression in hepatocarcinogenesis has not been confirmed. In the case of mouse epidermis, no such external stimulus is required, and preliminary investigations in our laboratory (J. Hully, Y. Dragan, and H. Pitot, unpublished observations) have demonstrated that par-

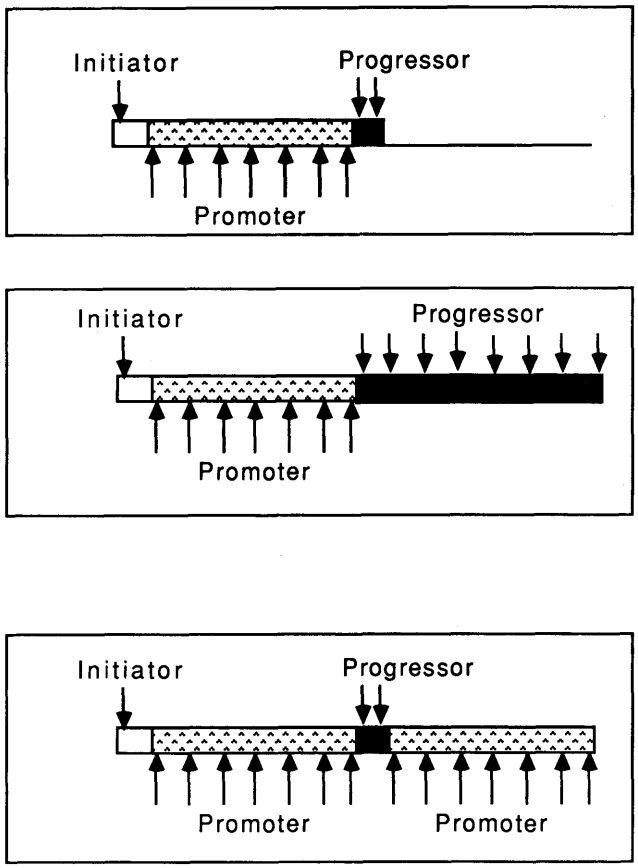

Figure 1. Models for testing potential progressor agents: initiation-promotion-progression. Figure shows format of test systems used for identification of progressor agents. The arrows indicate the single or multiple administrations of the initiator, promoter, and progressor agents. The exact time intervals for multiple administrations vary with the specific agent. See text for further details.

tial hepatectomy at the time of administration of the progressor agent is probably not necessary for multistage hepatocarcinogenesis. Presumably, the increased mitotic rate of hepatocytes in the stage of promotion is sufficient if cell division is required for the fixation or progression as it is for the stage of initiation (33). The methods that have been utilized to quantitate the extent of the stage of progression and of administered progressor agents are discussed in another paper in this Symposium issue (57).

The Stage of Progression and Progressor Agents in Carcinogenesis in the Human

Although the original basis for the "two-hit" hypothesis was developed from epidemiologic studies in the human (55), the clear demonstration of a defined stage of progression in human carcinogenesis is not so readily accomplished as in the experimental animal. However, the presence of premalignant and preneoplastic lesions pathologically demonstrable in a variety of human cancers is completely in concert with the stages of promotion and progression as components in the development of malignant human neoplasia from initiated cells (58, 59). Furthermore, just as the "focus-in-focus" lesion is characteristically noted in the development of the stage of progression during rat hepatocarcinogenesis (21), similar focal malignancies developing in benign lesions in the human have been reported for a variety of human neoplasms, both epithelial (60–63) and mesenchymal (59, 64, 65). Furthermore, careful study of a variety of specific human neoplasms from the earliest definable stages has demonstrated a series of morphologic and karyotypic changes that can be considered analogous to the model systems in multistage carcinogenesis in animals (66, 67). Therefore, there is ample evidence that the stage of progression can be defined in the development of human cancers. Such a concept raises the interesting idea that a number of human tumors may represent cells in the stage of promotion, such promotion being the result of the action of endogenous (16) or uncontrolled exogenous promoting agents. A partial list of such lesions is seen in Table III (68–79). Table III also indicates where evidence for the regression, spontaneous or otherwise, of such lesions has been demonstrated.

Identification of Progressor Agents Active in Human Carcinogenesis

The use of model systems such as those depicted in Figure 1 for the identification of progressor agents that may potentially be important in human carcinogenesis has not been generally accepted for a variety of reasons. On the other hand, one may make certain predictions about agents that potentially may act to induce the stage of progression either in their role as a complete carcinogen or as a progressor agent. Because of the unique characteristic of karyotypic abnormalities of cells in the stage of progression (24), one may propose that clastogenic agents are potentially progressor agents. Although it is clear that not all clastogens fall into this class, some of the known human carcinogens that in the past have defied attempts to demonstrate their carcinogenicity in animals are clastogenic. These include benzene, arsenicals, and asbestos (80). Presumably, such agents exert their progressor or clastogenic effects on rapidly growing cell populations, such as bone marrow in the case of benzene, to stimulate the fortuitous clastogenicity of exposed replicating cells. A synergy of promoter and clastogenic agents, which has been noted in multistage rat hepatocarcinogenesis (81), may also be inherent in the synergistic effect of tobacco smoking and asbestos exposure (82). Although the principal effect of tobacco smoking may be considered to

Table III. Human Lesions Postulated to Be in the Promotion Stage of their Development to Neoplasia

Lesion	Potential subsequent lesion(s)	Ref.
Aberrant colonic crypts	Adenomas and carcinomas	68
Adrenal cortical nodules	Adenomas and carcinomas	69
Dysplasia		
Cervix	Carcinoma *in situ* or regression	70
Esophagus and stomach	Carcinoma *in situ*	71
Larynx	Carcinoma *in situ*	72
Intestinal metaplasia of gastric mucosa	Carcinoma or gastric mucosa differentiation	73
Leukoplakia		
Bladder	Carcinoma or regression	74
Oral cavity	Carcinoma or regression	75
Liver cell adenoma	Carcinoma or regression	76, 77
Mesenteric fibromatosis	Uncertain	78
Pulmonary lymphangioleiomyomatosis	Uncertain	79

result from its complete carcinogenicity, the extended period and reversibility of its effects (83, 84) argue that its promoting action is the predominant factor in the development of malignant neoplasms. Thus, tobacco smoking sets the stage for the progressor action of asbestos. Arsenicals, which have been extremely difficult to demonstrate as carcinogenic in animals, presumably exert carcinogenic effects by their chronic clastogenic effects on replicating cell populations (epidermis) or cells in the stage of promotion in other organs (liver). This presumption is borne out by the report of Pershagen *et al.* (85), who found that smokers exposed to arsenic exhibited a 4- to 5-fold greater risk of developing lung cancer than individuals exposed either to cigarette smoke or to arsenic alone.

Both experimental and epidemiologic studies strongly support the concept that the development of neoplasia in the human involves the stages of initiation, promotion, and progression, as seen in animal systems. Since cancer develops in the final stage of neoplastic development, the risk of human exposure to progressor agents may be a significant factor in the development of specific human neoplasms. In view of the endogenous and ambient exposure to numerous promoting agents and the ubiquitous spontaneous initiation of human cells, progressor agents, some of which are as yet undetected, can pose a significant risk to the development of human cancer in many human populations.

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