S-10-1 Vitamins and Carcinogenesis: An Overview

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I. INTRODUCTION

Studies of the molecular biology of normal and tumor tissue have demonstrated that cancer is a disease of genetic regulation. The fundamental disorder in neoplasia is the expression of inappropriate genes which alter the phenotype of the transformed tissue and confer properties of abnormal growth, invasiveness and the potential for metastasis. Gene expression varies normally from conception to senescence as the organism changes to meet its challenges at each state of development. In general, the phenotype of tumors resembles fetal tissue more than adult tissue. Cancers can result from purely genetic determinants inherent in a given individual (like the absence of the retinoblastoma suppressor gene) or from a combination of environmental and hereditary factors that affect gene expression. The question which we are posing in this Symposium is "How can vitamins and biofactors alter the process of carcinogenesis to prevent or postpone the appearance of a malignant tumor?"

II. STAGES OF CARCINOGENESIS

Carcinogenesis is a multistage process which is characterized by initiation (alteration of DNA), promotion (cellular proliferation) and progression (karyotypic instability and frank malignancy). Pitot and Dragan [1] have described the stages of initiation and progression as irreversible, whereas the stage of promotion is reversible. The characteristics of these stages are summarized in Table 1.

The stage of initiation is not irreversible until the somatic mutation is fixed by DNA replication. In chemical carcinogenesis the very initial step is the reaction of DNA with electrophilic molecules to form adducts. Some carcinogens like dimethyl sulfate are electrophiles per se and can react directly with nitrogen atoms of the four bases that make up DNA. Others, like benzpyrene, a procarcinogen, must be oxidized to an electrophile by cytochrome P-450. Before initiation can occur, the chemical adduct formed must lead to a permanent change in DNA structure. This means it must survive the actions of factors which inhibit adduct formation, affect cytochrome P-450 activity or block the numerous enzymes which accomplish DNA repair. Ames and Gold [2] have claimed that the major source of DNA damage are endogenous oxidants that can result in as many as $10^7$ hits per cell per day in man and more in experimental animals. Although most of this damage is repaired, the cell injury promotes mitogenesis which in turn increases the probability that mutations will persist or that new ones will occur. Primary genotoxic carcinogens include alkyl imines, epoxides, mustards, cyclophosphamide, bis(chloromethyl) ether. Procarcinogens requiring activation include diethylnitrosamine, aflatoxin B1, 2-naphthylamine, 2-acetylaminofluorene, polycyclic hydrocarbons, 1,2-dimethyl-hydrazine and the mixture of hydrocarbons in tobacco smoke.

The stage of promotion is reversible and is environmentally modulated. Promoting agents increase the risk of cancer development by increasing the rate of proliferation of normal cells and by selectivity increasing the growth of initiated cells. Focal lesions resulting from initiated cells in the presence of a promoter may regress when the promoter is removed and recur when it is readmin-
TABLE 1. SOME CHARACTERISTICS OF THE STAGES IN CARCINOGENESIS

<table>
<thead>
<tr>
<th>STAGE</th>
<th>CHARACTERISTICS</th>
</tr>
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<tbody>
<tr>
<td>Initiation</td>
<td>Irreversible</td>
</tr>
<tr>
<td></td>
<td>Requires fixation</td>
</tr>
<tr>
<td></td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>No measurable threshold</td>
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<tr>
<td>Promotion</td>
<td>Reversible</td>
</tr>
<tr>
<td></td>
<td>Environmentally modulated</td>
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<tr>
<td></td>
<td>Maximal response</td>
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<tr>
<td></td>
<td>Threshold</td>
</tr>
<tr>
<td>Progression</td>
<td>Irreversible</td>
</tr>
<tr>
<td></td>
<td>Somatic aneuploidy</td>
</tr>
<tr>
<td></td>
<td>Progressive karyotypic instability</td>
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Promotion is continually modulated by environmental factors such as the composition and the amount of the diet. It is suspected in fact, that the effect of vitamins and biofactors in reducing the incidence of neoplasia in experimental animals is principally due to the inhibition of promotion. Some agents which have been identified as promoters in experimental carcinogenesis include ultraviolet radiation, phenobarbital, phorbol esters, dietary fat, ethanol and a series of hormones including prolactin, estrogens and androgens. Since the stage of promotion often involves the activation of receptors, it has a threshold and a maximum response. It is believed, furthermore, that promoting agents play a major role in the development of many human cancers including those of the lung, breast, prostate and gastrointestinal tract.

The stage of progression is characterized by karyotypic instability and the development of irreversible, aneuploid malignant cells. Chromosomal transpositions and other changes may occur which alter the genomic environment and result in marked changes in growth rate, invasiveness, metastatic capability and membrane composition. It is thought that oncogene expression may be a significant factor in the development of progression since oncogenic retroviruses are potent stimulators of the progressive stage of carcinogenesis. It is also known that karyotypic instability is associated with enhanced oncogene expression. Clastogens such as benzene, asbestos, arsenite and the herpes virus, as well as complete carcinogens, act as progressors.

III. THE ROLE OF ONCOGENES

Oncogenes were first discovered in retroviruses which cause tumors in birds and rodents and can transform animal cells in vitro [3]. It was then discovered by Bishop [4] that these viral oncogenes are related to precursor cellular genes (proto-oncogenes) which are conserved in evolution and were picked up by the retroviruses in the course of viral infection. Most proto-oncogenes encode proteins which are involved in the series of events by which growth factors control normal cell division. Oncogenes differ from their parent proto-oncogenes by one or more mutations which may be caused by external carcinogens. Oncogenes, of which more than 50 are known, encode proteins that fall into several classes: growth factors (sis), growth factor receptors (erb B, fms and kit), transducers of growth factor responses (src, ras, raf) and transcription factors (jun, fos, myc, myb, ski and ets). Several of these oncogene products are tyrosine-specific protein kinases. Some of these kinases are growth factor receptors and some are
cytosolic. The ras oncogenes are GTP-binding proteins and *sis* codes for a protein homologous with platelet derived growth factor. Thus, oncogenes which are derived from genes which orchestrate cellular division and growth, uncouple these activities from normal physiological controls. Oncogenes are expressed in both animal and human tumors and undoubtedly play a significant role in the promotion and progression of malignant tumors.

IV. ROLE OF VITAMINS AND BIOFACTORS IN CARCINOGENESIS

The vitamins and biofactors which have been implicated in chemoprevention of cancer can be divided into three classes; those affecting 1) gene expression (vitamin A and other retinoids and vitamin D), 2) DNA synthesis (folic acid and vitamin B12 and 3) antioxidation (carotenoids and vitamins C and E).

The structures of genomic receptors belonging to the steroid-thyroid-vitamin superfamily are shown in Fig. 1. The active form of vitamin A (all trans-retinoic acid) and vitamin D (1,25(OH)2D3) are ligands for 2 of these receptors. When the hormone or vitamin combines with its receptor the conformation of the receptor is altered which promotes DNA binding and affects gene expression [5]. Although all transretinoic acid is effective in inhibiting tumor development in some systems (skin, lymphopoietic cells and bladder) other retinoids are not convertible to retinoic acid (deoxyretinol and retinamines) exert chemopreventive activity against breast cancer. Experimental lung tumors are particularly resistant to retinoids. Since the genomic receptor for retinoic acid recognizes only the acid it follows that another pathway must be invoked to account for the activity of some retinoids [6].

![Figure 1. Structures of human genomic receptors for glucocorticoids (hGR) retinoic acid (hRR), thyroid hormone (hT3R) and vitamin D hormone (hVDR). The amino acid content of each receptor is shown by numbers at the right end of each bar. The receptors are aligned to show the constancy of the highly conserved DNA-binding domain in the middle (65 amino acids). The enhancer domain is at the N-terminal portion of each receptor and is variable. The ligand-binding domain is at the C-terminal end of each receptor and variable.](attachment:figure_1.png)

Vitamin D not only induces a calcium transport system in the gut and kidney, but also stimulates osteoblasts to produce alkaline phosphatase and osteocalcin. It can also stimulate mononuclear cells to differentiate into macrophages. Effects on oncogene expression and immunity have also been demonstrated in animals. Vitamin D and calcium have appeared to exert a protective effect against colorectal cancer in humans [7].
Folate and vitamin B₁₂ are essential for thymidine and hence DNA synthesis. It has been reported that they combat uterine cervical dysplasia.

The antioxidants, β-carotene, vitamin C and vitamin E have been observed to inhibit the incidence of various cancers under experimental conditions. β-Carotene is emerging as a unique antioxidant which can neutralize singlet oxygen at low oxygen tensions and appears to have an array of anti-cancer activities. These include inhibition of U.V. and chemically-induced tumors in rodents, decreased sister chromatid exchange and malignant transformation in cultured cells, enhanced immunity in cells in vitro and decreased bacterial revertants in mutagenesis assays [8]. These actions of β-carotene do not appear to be the result of conversion to retinal retinol since canthaxanthin, a diketo derivative of β-carotene without vitamin A activity, has approximately the same chemopreventive effect. Lycopene and phytoene are inactive.

Vitamin C and vitamin E (α-tocopherol) show some promise as chemopreventive agents but their activity and the range of tumors affected are less impressive than some of the other compounds discussed.

V. CLINICAL TRIALS OF CHEMOPREVENTION BY VITAMINS

Although chemoprevention of human cancer is a goal of public health practice only clinical trials can decide the effectiveness and safety of vitamins and biofactors. Some early results involving small numbers of subjects seem promising but further study is needed. Fifteen clinical trials supported by the National Cancer Institute of the U.S. are now in progress. Their results will be awaited with great interest.

SUMMARY

The pathophysiology of carcinogenesis as a multistage process has been reviewed and the rationale for the action of vitamins and biofactors has been presented. This overview will now be followed by presentations of the actions of retinoids, carotenoids, vitamin D and marine natural products by other members of this Symposium.

REFERENCES