# Properties of Intraepithelial Neoplasia Relevant to Cancer Chemoprevention and to the Development of Surrogate End Points for Clinical Trials (44165)

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*Abstract.* **Cancer chemoprevention is defined as the prevention of cancer by the administration of diet supplements or drugs. A drug discovery effort should therefore focus on finding agents that will avert the process of intraepithelial neoplasia which precedes invasive cancer. Over 30 agents developed by the chemoprevention program at the National Cancer Institue are being tested against intraepithelial neoplasia of many organ sites in more than 80 clinical trials. Two basic mechanisms underlie the onset and development of intraepithelial neoplasia. First is the development of the two precursor lesions of chronic diffuse epithelial hyperplasia and genomic instability, the latter being produced by "mutator" mutations in genes responsible for genomic stability, by gene copy amplification or loss from DNA breakage-fusion-anaphase-bridge cycles, by unequal sister chromatid exchange, and by accumulation of double minutes. Second is the development of multicentric intraepithelial neoplastic lesions which independently progress through each of the following processes at a continuously accelerating rate: clonal evolution, hyperproliferation, production of genomic structural variants, and apoptosis. Recommended chemoprevention strategies based on these mechanisms are (i) the development of better technology for early diagnosis, (ii) the development of multiple agents that block intralesional proliferation at steps along the signal pathway of mitotic signal transduction and along the signal pathway of synthesis of daughter cell components, (iii) the development of nontoxic anti-inflammatory agents, anitoxidants, antimutagens, and proapoptotics, (iv) the avoidance of "clonal escape" through use of drug combinations, and (v) the use of computer-assisted quantitative image analysis to assay modulation of surrogate end points in chemoprevention clinical trials.** [P.S.E.B.M. 1997, Vol 216]

Cancer chemoprevention is the prevention of cancer by the administration of diet supplements or drugs. At the tissue level, this amounts to preventing the onset or progression of neoplasia while it is still confined to the intraepithelial compartment and has not yet become invasive (viz., intraepithelial neoplasia). The chemoprevention program of the National Cancer Institute includes information searches of the international literature, drug screening in cell culture and animal models, preclinical toxicology testing in animals, and toxicity and efficacy testing in human trials (1). Table I outlines the scope of the chemoprevention program, giving the ABCs of drug development: chemoprevention *a*gents, *b*iomarkers for use as end points in clinical trials, and human *c*ohorts with preinvasive neoplastic disease suitable as subjects for clinical trials. Over 30 agents are being tested singly and in combination in over 80 clinical trials, and another 10 agents are nearing the end of preclinical testing. A central aim of the chemoprevention program is to learn as much as possible about the molecular and cellular mechanisms of intraepithelial neoplasia and to use this information to plan directions with the best chance of success. The contents of this strategy are described below.

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**Table I.** Chemoprevention: Agents, Biomarkers, and Cohorts

| | Agents classified by mechanism | Intermediate biomarkers | Clinical cohorts (Phase II) | Clinical cohorts (Phase III) |
|---|---|---|---|---|
| Colorectal | Antiinflammatories (sulindac, piroxicam, aspirin, ibuprofen)<br>Antiproliferatives (DFMO, calcium, curcumin) | Adenomas, proliferative indices, aberrant crypts, Lewis blood group antigens, sialyl-Tn antigen | Patients with previous adenomas or with adenomas <1 cm in diameter | Subjects at high risk (family history of adenomas or colorectal cancer, previously treated breast or endometrial cancer) |
| Prostate | Testosterone 5α-reductase inhibitors (finasteride)<br>Retinoids (4-HPR)<br>Antiproliferatives (DFMO)<br>*ras* farnesylation inhibitors (*d*-limonene) | PIN, PSA, PAP[a], cytokeratins (loss of 50–64 kDa), vimentin, nucleolar prominence, DNA content | Patients with PIN without prostatic adenocarcinoma; patients scheduled for radical prostatectomy | Patients with elevated serum PSA; subjects ⩾60 years of age |
| Lung | Retinoids/carotenoids (Vitamin A, 13-*cis*-retinoic acid, β-carotene)<br>Dithiolthiones and other organosulfur compounds (Oltipraz, *N*-acetyl-L-cysteine) | Cellular atypia/ dysplasia in sputum, bronchial atypical metaplasia/dysplasia, PCNA, blood group antigens, *p53* | Patients with recently resected stage I lung or laryngeal cancer | Patients with previous lung, head or neck cancers; subjects at high risk (smokers, occupational exposure to asbestos) |
| Breast | Antiestrogens (tamoxifen, toremifene)<br>Retinoids (4-HPR)<br>*ras* farnesylation inhibitors (perillyl alcohol, *d*-limonene) | Atypical hyperplasia, DCIS, LCIS[b] | Patients scheduled for breast cancer surgery | Patients with previously treated breast cancer |
| Bladder | Antiinflammatories (sulindac, piroxicam, aspirin, ibuprofen)<br>Antiproliferatives (DFMO)<br>Retinoids (4-HPR) | TIS, dysplasia, DNA content, F- and G-actins, integrins, loss of heterozygosity (e.g., 9q), blood group antigens, *Rb* | Patients with previously resected TIS or $T_a$, $T_1$ disease without TIS | Subjects at high risk (occupational exposure to aromatic amines) |
| Oral | Retinoids/carotenoids (vitamin A, 13-*cis*-retinoic acid, β-carotene)<br>Antiinflammatories (carbenoxolone) | Dysplastic leukoplakia, keratin expression, GGT[c] | Patients with dysplastic leukoplakia | Patients with previously treated head and neck cancers; subjects at high risk (smokers, tobacco chewers) |
| Cervix | Retinoids (vitamin A, 4-HPR)<br>Antiproliferatives (DFMO)<br>Folic acid | CIN[d] | HPV-negative patients with CIN III | Patients with CIN |

[a] PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; PAP, prostatic alkaline phosphatase.
[b] DCIS, ductal carcinoma *in situ;* LCIS, lobular carcinoma *in situ.*
[c] GGT, γ-glutamyl transpeptidase.
[d] CIN, cervical intraepithelial neoplasia.

## The Onset of Intraepithelial Neoplasia

**The General Nature of Epithelia.** It is useful to summarize in general terms the nature of the epithelial cell sheets from which human carcinomas derive. With dimensions resembling a sheet of writing paper (i.e., up to 0.1 mm in thickness [2–10 epithelial cells] and with a broad surface area), epithelial cell sheets cover the external surfaces or line the cavities and ducts of the organ systems of the body, including (i) skin and mucous membranes; (ii) nasopharynx, larynx, and lung; (iii) oral cavity, esophagus, and intestinal tract; (iv) kidney pelvis, ureters, and bladder; and (v) reproductive system (fallopian tubes, uterus, and urethra). From these epithelial sheets are derived a variety of glandular epithelia with specialized secretory or absorptive functions, including in particular breast and prostate. Epithelial cell sheets, whether squamous or glandular, generally exhibit a

self-renewing transition pathway through three cell layers of the following general types: a basal layer of proliferating stem cells, an intermeidate layer of nondividing differentiated cells, and a superficial layer of mature cells which eventually undergo apoptosis and are shed to the external environment either directly or by way of a communicating lumen or tract. The estimated rate of self-renewal among squamous and glandular epithelia in rodents generally varies from 2 to 10 days, depending on their location and function (2).

**The Formation of an Intraepithelial Neoplasm as the Result of Mutational Changes in Proto-Oncogenes and Tumor Suppressor Genes.** Against a background of genomic instability (i.e., an abnormally increased rate of unrepaired DNA breakage with formation of abnormal genomic structural variants), mutations of some 50 oncogenes and a larger number of tumor suppressor genes with loss of heterozygosity may cause an epithelial cell to undergo neoplastic clonal expansion (neoplasia is defined in the next section) by means of three basic mechanisms: (i) a block in the differentiation pathway with continuing production of stem cells, (ii) hyperproliferation, and (iii) a block in normal apoptosis. These mechanisms, individually described below, most commonly occur together and cooperate synergistically (3).

**Initial Clonal Expansion Heralds the Onset of Intraepithelial Neoplasia.** Against a background of genomic instability within an epithelium, the onset of neoplasia is heralded by the appearance of one or more clonal expansions of genetically altered cells, as illustrated in Figure 1.

*Mutational block in a differentiation pathway.* It is instructive to appreciate that although initial neoplastic clonal expansions are most often abnormally hyperproliferative (4), they need not be. A mutational block in the pathway of transition from a proliferating basal stem cell to a nonproliferating differentiated cell (e.g., mutation of the *mad* gene, whose protein product forms a complex with the *myc* protein which switches on the differentiation pathway in epithelia [5]) would force the clonal buildup of a mass of basal cells which would continue to proliferate at normal, or even subnormal, rates. This clonal mass, illustrated in Figure 1, provides the simplest definition of a neoplasm at its onset: a continuing focal, clonal accumulation of cells that exhibits disorganized architecture, an absence of normal maturation, and a tendency to compress (or distort) surrounding normal cells. Prior to the onset of invasiveness across the epithelial basement membrane, intraepithelial neoplasia is classified as benign. After invasiveness occurs, it is diagnosed as malignant or as cancer.

*Mutational change in a proliferation-related signal pathway.* Cell proliferation is controlled by the cell surface and cytoplasmic networks of signal pathways mediated by protein kinases that are frequently the gene products of proto-oncogenes and tumor suppressor genes [e.g., growth factors and their receptors (EGFR, *erb* B-2, TGFα, IGFR, etc.), and the *ras/raf*/MAP/MEK kinase pathway (6)]. A basal stem cell that sustains a mutation resulting in constitutive signaling from a growth factor receptor will undergo uncontrolled hyperproliferation (3). In this case, differentiation will continue to produce intermediate and superficial cells. As an example, intraepithelial, preinvasive squamous-cell neoplasms of the head and neck characteristically show hyperproliferation with continuing differentiation, forming hyperkeratotic surface layers and abnormal centers of keratinizing cells, or even individually keratinizing cells, within the substance of the neoplastic cell mass (7). These differentiated structures stain positively for expression of the *mad* gene (8).

*Mutational block in apoptosis.* Self-renewing epithelia control inappropriate oversizing of their basal stem-cell pools by programmed cell death, or apoptosis. The *Bcl-2* proto-oncogene product is a 26-kDa protein which suppresses apoptosis. Hyperexpression of the *Bcl-2* gene in a basal stem cell, whether it is produced by gene amplification or occurs secondary to mutations in other genes, blocks apoptosis, which results in the production of an expanding mass of basal stem cells that continue their normal rate of proliferation and differentiation. The mass qualifies as a benign neoplasm because of its focality, its lack of known

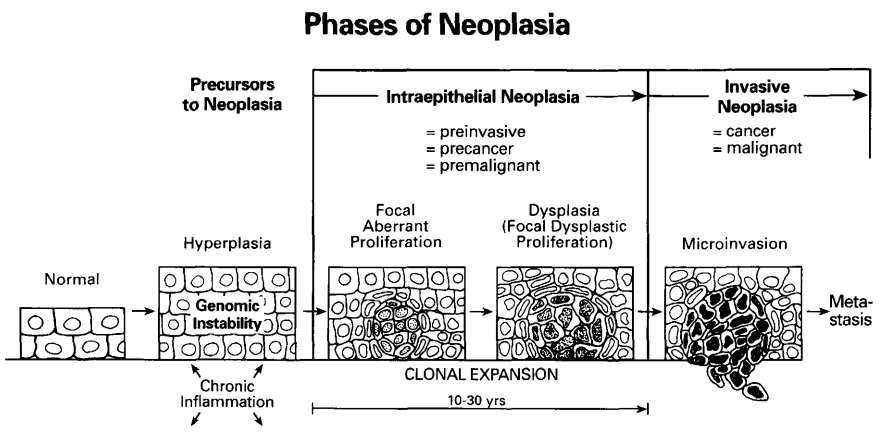


**Figure 1.** Overview of preinvasive neoplastic progression. The appearance of a monoclonal expansion marks the onset of intraepithelial neoplasia. The average time between onset of a visible neoplastic lesion within the epithelium and invasive neoplasia is many years.

function, the disorderliness of its cells, and especially because its borders tend to compress or distort adjacent normal tissue. Hyperplastic polyps of the colon, as distinguished from adenomatous polyps, show very few mitoses and markedly increased staining with monoclonal antibody to *Bcl-2* protein (9), indicating that they arise because of a block in the normal rate of apoptosis. Hyperplastic polyps are classified as benign neoplasms without malignant potential. Current evidence is strong that the *Bcl-2* protein, attached to the outer membrane of mitochondria, the endoplasmic reticulum, and the nuclear membrane, through interaction with Bax protein, may be part of an apoptotic control mechanism which regulates the stimulation of programed cell death by reactive oxygen species (10).

**The Distinction between the Growth Rate of a Neoplasm (Net Rate of Increase in Total Bulk) and the Proliferation Rate of Its Individual Cells.** The overall growth rate of a neoplasm (i.e., the rate of increase in its total bulk) is the net difference between the cycling rate of its cells and the sum of two other rates: the rate of exit from the cell cycle to begin differentiating and the rate of apoptosis. The genomic instability that gives rise to cell variants which grow faster and contribute to clonal evolution (see below) also gives rise to variants that do not survive cell-cycle checkpoints for DNA damage and undergo apoptosis. As long ago as 1953, Willis (11) remarked on the enigma that basal cell carcinomas of the skin show frequent mitoses, yet increase in size very slowly. Given a basal cell carcinoma with a mitotic index of 1% and a mitotic duration of 1 h, and that all of its cells remain viable and continue to cycle, its total bulk should double in half a day. Yet the reported time for the average basal cell carcinoma to double in bulk averages 26 days (4). This discrepancy has been accounted for by the finding that basal cell carcinoma cells undergo apoptosis at a rate that approaches their rate of proliferation (12).

## The Diagnostic Terminology of Intraepithelial Neoplasia Used by Pathologists

Figures 2 and 3 illustrate the development of intraepithelial neoplasia in squamous epithelium such as cervix and in glandular epithelium such as colon, and also present the terminology used by pathologists to describe various diagnostic features. A very important point is that the morphology of neoplastic cells just prior to invasion, when they are described by relatively benign terms such as severe atypia, dysplasia, and severe intraepithelial neoplasia, differs little if at all from their morphology just after invasion, although the terms describing them change dramatically to the dreaded diagnoses of carcinoma, cancer, and malignancy. As shown in Figures 1–3, the onset of neoplastic disease appears at least a decade before the phase of invasiveness. It is misleading if not potentially harmful to the patient to think that the condition of intraepithelial neoplasia is fundamentally different from that of cancer, and that it requires some kind of conversion to become a malignancy. This

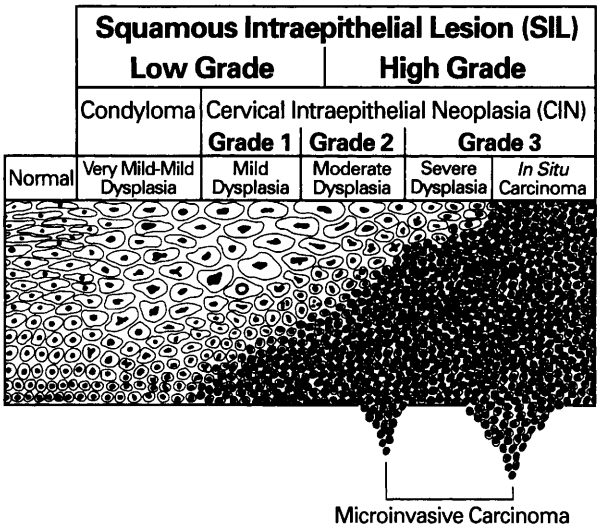


**Figure 2.** Diagram of cervical intraepithelial neoplasia (CIN), with three grades, more recently reclassified as squamous intraepithelial lesion (SIL), with two grades. The borderline between inflammatory hyperplasia and early intraepithelial neoplasia is not clear-cut. The diagnosis of low-grade SIL may include some cases of "reactive" hyperplasia, but the diagnosis of high-grade SIL includes only cases of neoplastic change.

erroneous belief is abetted when intraepithelial neoplasia is called by a name that is confounding as well as misleading, preneoplasia (alluding to intraepithelial *neoplasia* as *pre*-neoplasia is oxymoronic). In 1969, before the term intraepithelial neoplasia came into use, Foulds (13) stated: "The most frustrating gap in the terminology of pathology of tumours in man is a lack of a satisfactory name for the so-called precancerous lesions. 'Preneoplastic' . . . can only mean that the lesions are not neoplastic whereas I maintain strongly that they are neoplastic and that this should be recognized in their designation."

The diagnosis of carcinoma *in situ* is frequently given to intraepithelial neoplastic lesions that show extensive replacement of the normal epithelium by neoplastic cells in a pattern that is judged by the pathologist to have a higher risk of early invasion that severe intraepithelial neoplasia. The diagnosis of carcinoma *in situ* cannot be made with the same assurance and certainty as can invasive carcinoma, because it does not have an irrefutable marker as good as invasiveness. There is now well-reviewed evidence that severe dysplasia and carcinoma *in situ* are virtually identical and form a single continuum of neoplastic change (14).

In Figure 3, the colon crypt is seen to be a test tube–shaped downward vertical extension of the single-layered surface columnar epithelium which faces the fecal stream. In histological sections perpendicular to the surface epithelium, the crypts appear as "a row of test tubes." The two side walls of a crypt (called hemicrypts) each support an average of 40 contiguous columnar epithelial cells extending from the base to the mouth of the crypt, beyond which the epithelial cell layer opens out on, and becomes continuous with, the surface epithelium. Cells in the lower third of the crypts are mostly proliferating basal cells, and in the

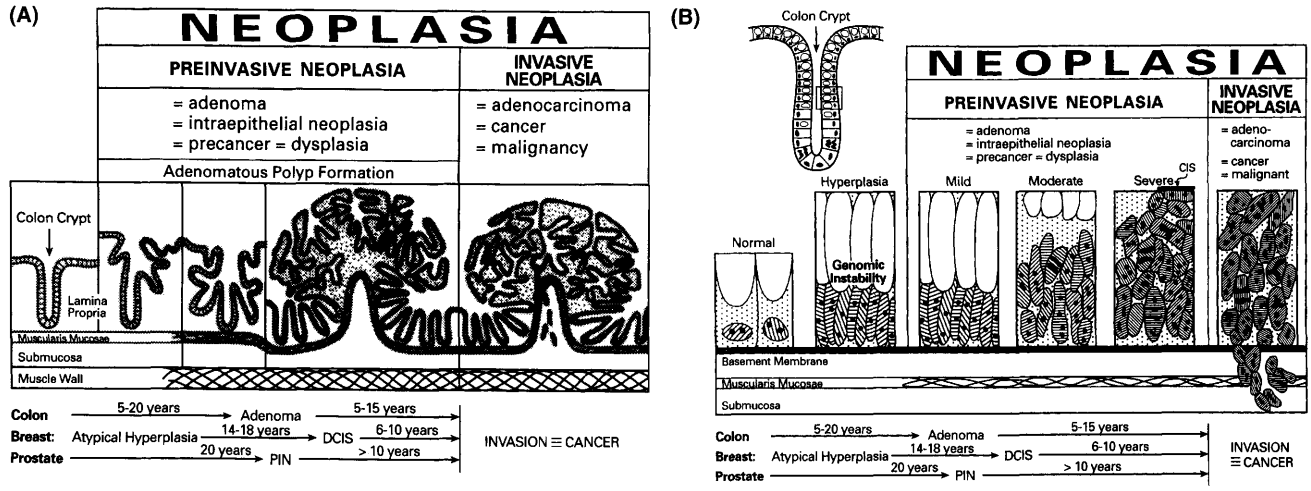


**Figure 3.** (A) Diagram of colorectal polyp formation at low magnification. Uncontrolled clonal expansion of a cell in the upper portion of the crypt leads to formation of a mass of multiple abnormal crypts that bulges up to form a polyp with a core made up of submucosal blood vessels. (B) Diagram of colorectal polyp formation at high magnification. The cell nuclei of intraepithelial neoplasia of glandular epithelium on the surface of the polyp show the same aberrant variation in size, shape, and chromatin texture as the cell nuclei of intraepithelial neoplasia of squamous epithelium.

upper two-thirds they are a mixture of proliferating and nonproliferating cells differentiating into absorptive cells, which then undergo apoptosis and shed into the fecal stream. Viewed collectively, the crypt basal, intermediate, and superficial cells are analogous to the basal, intermediate, and superficial cells of squamous epithelia described previously. By convention, the diagnosis of invasive neoplasia (adenocarinoma) of the colon is not made until the neoplastic cells have invaded not only across the basement membrane of the crypt epithelium, but also across the subjacent muscularis mucosae (Fig. 3B).

## Chronic Diffuse Epithelial Hyperplasia: A Common Initial Precursor of Intraepithelial Neoplasia

Chronic diffuse epithelial hyperplasia is commonly seen as the precursor to intraepithelial neoplasia. Probably the most frequent cause is stimulation by growth factors and proliferation-inducing reactive oxygen species produced by the lymphocytes and macrophages of chronic inflammatory infiltrates in the subepithelial stroma. For example, in the oral mucosa, subepithelial chronic inflammatory cells were shown to induce EGF and EGF receptors in the overlying squamous epithelium (15). Examples of chronic inflammation associated with the neoplastic process are ulcerative colitis (16), urinary bladder inflammation associated with schistosomiasis (17), stones (18),and long-term indwelling catheters (19), gall bladder inflammation secondary to stones (20), and Barrett's esophagus, a condition in which esophageal inflammation secondary to gastric acid reflux leads to hyperproliferative metaplasia of the esophageal epithelium (from squamous type to hyperproliferating intestinal epithelial type), which progresses to intraepithelial neoplasia (21). In the skin, actinic (solar) keratosis, the most common type of intraepithelial neoplasia, is practically always associated with chronic inflammation in the subepidermis (22). In the larynx, subepithelial inflammation is a significant predictor of progression to carcinoma (23). Cigarette smoke produces chronic inflammation of the respiratory mucosa and induces metaplasia of the ciliated secretory epithelium to stratified squamous type, from which intraepithelial neoplasia develops (24).

Another cause of epithelial hyperproliferation is the regenerative hyperplasia associated with exposure to cytotoxic chemicals (25). The importance of hyperproliferation to the neoplastic process has been reviewed (26, 27) and probably has never been better defended than in a recent series of lively and informative interchanges between Farber (28) and Stemmermann et al. (29).

## Genomic Instability: An Essential Precursor of Intraepithelial Neoplasia

Genomic instability refers to chronically increased rates of unrepaired DNA breakage with formation of abnormal structural changes in the genome. According to long-established usage, the term mutation refers to alterations of the DNA sequence within one gene, ranging in extent from a single nucleotide to a few kilobases. In this article, permanent and heritable structural changes of the genome of all sizes, from point mutations to loss of part of a chromosome arm containing thousands of genes, or even to stable loss or gain of an entire chromosome (e.g., Turner's syndrome and Down syndrome, respectively), will be referred to as genomic structural variations, including those that may be heritable only to the next cell generation before they again become modified in S phase. Genomic structural variations occur at three levels of DNA organization: at the level of the primary DNA sequence (classic mutations involving single nucleotides and oligonucleotide sequences within a gene), at the level of DNA segments containing many genes (am-

plicons showing gain, loss, or recombination), and at the level of whole chromosomes (karyotypic aberrations of chromosome structure and number).

## Mechanisms of Genomic Instability

**Mutator Mutations in Genomic Stability Genes.** A paradigm to explain the generation of genomic instability has been offered by Loeb (30). Genes whose expression is required to maintain the fidelity of duplicating and segregating the genome are called genomic stability genes. Mutations in one or more of these genes, aptly termed mutator mutations, lead to genomic instability. The list of genes whose functions help maintain genomic stability is long (30), including gene functions concerned with the following: (i) synthesis of balanced nucleotide pools (31), (ii) DNA replicaiton, (iii) repair of damaged DNA, (iv) cell-cycle checkpoints preventing survival of cells with excessive DNA damage, (v) genes controlling the apparatus for synthesis and coordination of cell division and DNA segregatin (DNA condensation, centriole synthesis and movement, spindle fiber and kinetochore synthesis, chromosome alignment at metaphase and anaphase, and cytokinesis), (vi) genes coding for xenobiotic metabolizing enzymes which catalyze scavenging of activated carcinogens and reactive oxygen species before they can damage DNA. A conservative estimate of the minimum number of genomic stability genes would be in the low hundreds. Since the genomic instability produced by mutation of a genomic stability gene undeniably contributes to the risk of neoplasia, genomic stability genes are ready candidates to become tumor suppressor genes.

**Gene Amplification and Loss of Heterozygosity Produced by DNA Strand Breakage with Aberrant Recombination.** Figure 4 illustrates the principle mechanisms of gene amplification and loss of heterozygosity, which are as follows.

*Gene amplification produced by the "breakage-fusion-bridge cycle."* As shown in Figure 4A, a postreplication (late S or G2) double-stranded DNA break in one sister chromatid produces a proximal short arm and a distal acentric fragment having different destinies. At cell division, the shortened broken chromatid segregates to a daughter cell in G1 phase and is replicated in S phase up to the "sticky ends" of the break, which fuse together to produce a dicentric chromosome. Sticky ends refer to the fact that the two nucleotide sequences at the end of a double-stranded break in the DNA double helix overhang each other by a few nucleotides and tend to fuse readily with other sticky ends that have complementary nucleotide sequences. Breaks in microsatellite DNA sequences are especially likely to fuse because, being made up of 50–200 dinucleotide repeats in tandem, they are very likely to have complementary sticky ends. When a dicentric produced by fused sister chromatids forms an anaphase bridge, breakage does not occur at the original fusion point, but randomly at

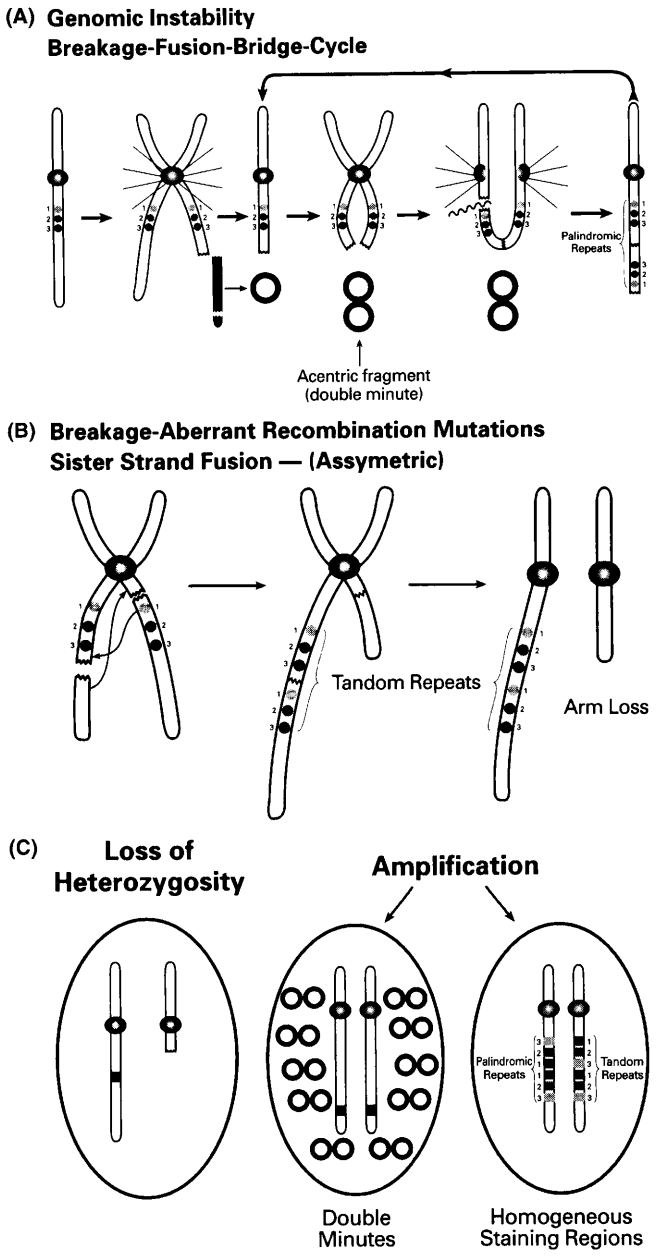


**Figure 4.** (A) The breakage-fusion-bridge cycle. A broken chromosome segment forms an acentric ring leaving a short chromosome arm. During growth cycling the broken ends repeatedly fuse and form an anaphase bridge with breaks at different locations. The acentric ring replicates to form a double ring, which continues replicating with each cell cycle in this form. Microscopically in a metaphase spread the palindromic gene repeat segments appear as homogenous staining regions, or HSR, and the double rings appear as double minutes. (B) Asymmetric sister strand fusion. Sister chromatid breaks at different locations transpose to form a long chromatid with tandem gene segment repeats and a short chromatid with gene loss. With many repetitions of this process, the tandem gene segments appear as homogenous staining regions, or HSR. (C) Summary of mechanisms of gene amplification and loss of heterozygosity. If multiple gene copies in double minutes or on HSR favors escape from growth controls, these cells accumulate. If loss of an allele uncovers a mutated tumor suppressor gene, these cells will accumulate.

any point, segregating a short chromosome with lost genes and a long chromosome with extra gene copies. The long chromosome segregates to a daughter cell and replicates in S phase to its sticky ends, which fuse as before to create a dicentric that forms another anaphase bridge. This "breakage-fusion-bridge cycle" continues indefinitely, producing a chromosome with many palindromic (nose-to-nose) gene repeats. The site of these many repeats can usually be visualized on metaphase chromosomes as a homogeneous staining region, or HSR (32, 33).

*Gene amplification produced by unequal sister chromatid exchange.* As shown in Figure 4B, if multiple DNA double-stranded breaks occur in both sister chromatids being formed during S phase, unequal sister chromatid exchange may occur such that the proximal sticky end of a break near the telomere may fuse with the distal sticky end of a break near the centromere, producing a very long chromatid with gene repeats in tandom (nose to tail). Many repetitions of such unequal sister chromatid exchanges produce regions of amplified gene dosage in which the gene repeats are arranged in tandem, as opposed to palindromic, sequence. The site of these many tandom chromatid segments also forms homogeneous staining regions, or HSR, on metaphase chromosomes (32).

*Gene amplification produced by accumulation of distal chromosome fragments circularized into "double minutes."* As shown in Figure 4C, after double-stranded DNA breaks occur, acentric fragments are produced. Such fragments may contain a telomere if the break occurred at the end of a chromosome arm, or not, if they derive from an interstitial region between two double-stranded breaks on the same chromatid arm. Acentric fragments tend to fuse their ends and circularize. During S phase, they replicate synchronously with the rest of the genome to form "double minutes," so called because they appear as tiny paired structures within a metaphase spread of chromosomes. Because double minutes have no centromere, they segregate randomly and unequally at mitosis. If a double minute contains genes of selective advantage to the daughter cell because they permit escape from growth controls, cells will tend to accumulate that have many such double minutes, amplifying the number of growth enhancing genes, as shown in Figure 4C. Acentric circular DNA fragments tend to reintegrate into genomic DNA (3, 34).

*Loss of heterozygosity produced by accumulation of chromosomes with lost arm segments (allelic loss).* With each of the three mechanisms of allelic amplification described above, chromosomes or chromatids with lost arm segments are produced that segregate to daughter cells. If the chromosome with a lost arm segment is of selective advantage to a daughter cell in terms of escape from growth controls, particularly if the lost segment contains a tumor suppressor gene, such cells will tend to accumulate to form a population exhibiting loss of heterozygosity (Fig. 4C).

## Characteristics of Intraepithelial Neoplasia Important to the Development of Chemoprevention Strategies

**Multicentricity: Intraepithelial Neoplasia Develops at Multiple Sites That Progress Independently.** Exposure of epithelial cell sheets to carcinogens usually occurs over a broad area, simultaneously exposing millions of proliferating basal cells to the risk of genotoxicity and postnecrotic regenerative proliferation. The skin, for example, has approximately one million basal cells per square centimeter; DNA-damaging solar radiation reaching an area of skin measuring 10 by 10 centimeters would simultaneously expose about a hundred million basal cells to the mutational and DNA breakage effects of pyrimidine dimerization and reactive oxygen species. Carcinogens absorbed into the body from the respiratory tract, such as those in cigarette smoke (35), or the digestive tract, such as nitrosamines derived from nitrite in unrefrigerated vegetables (36), are delivered *via* diffusion from subepithelial capillary networks to broad areas of epithelia, again simultaneously exposing millions of proliferating basal cells to DNA modification and mutation.

It is little wonder, then, that neoplastic clonal expansions start at more than one site in an epithelium that has developed diffuse genomic instability because of chronic exposure to carcinogens (Fig. 5). Slaughter (37), in a landmark paper describing observations on 783 patients with oral cancer, was the first to focus attention on the multicentric origin of *in situ* neoplasms of the oral cavity, each progressing independently to squamous-cell carcinoma. He coined the term "field cancerization" to describe an epithelium preconditioned by a carcinogenic agent. Over 11% of Slaughter's patients simultaneously exhibited one or more independent squamous-cell carcinomas involving the esophagus or lung. His concept of field cancerization of an epithelium may now be understood as the development of diffuse genomic instability after prolonged carcinogen exposure, with development of neoplastic lesions at multiple sites. Multiple actinic (solar) keratoses of the face is a common example of the multicentric development of intraepithelial neoplastic lesions. In one study (38), 8 of 15 actinic

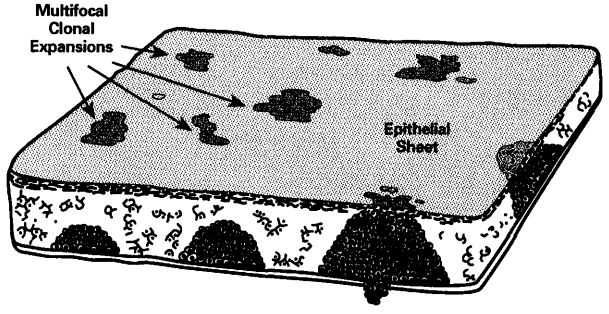


**Figure 5.** Intraepithelial neoplasia develops at multiple sites which progress independently, in an epithelium subject to diffuse genomic instability (called field cancerization by Slaughter [37]).

keratoses exhibited *p53* gene mutations, and in another (39), 12 of 26 actinic keratoses showed overexpression of cyclin D and p53 protein. Adenomatous polyps of the colon are another example of multicentric intraepithelial neoplastic lesions, this time lifted up from the mucosal surface on fibrovascular stalks. As is now well known, adenomatous polyps exhibit different numbers and combinations of genetic lesions, not only among different polyps, but also at different sites within the same polyp (40). As a final example, in the same prostate multicentric lesions of prostatic intraepithelial neoplasia were each shown to contain different patterns of aneuploidy (41).

**Accelerating Clonal Evolution.** Clonal evolution of neoplasms, first enunciated by Nowell (42, 43), has been reviewed in relation to intraepithelial neoplasia previously (44). Briefly, it is the continuous occurrence within a neoplastic population of genomic structural variants which undergo clonal expansion at a more rapid rate of proliferation than surrounding cells. Further clonal expansions of variant cells may occur within the same original expanding clone, or at other sites in the neoplastic population within independent lines of clonal evolution. Thus, at any given time, multiple clonal expansions may occur at different sites in the same tumor. The Gleason score, constructed by the pathologist to indicate the aggressive potential of prostate cancers, is the sum of two numerical grades given to the least differentiated and most differentiated regional patterns seen. It is illuminating to appreciate that the two regional patterns represent the phenotypic expression of separate lines of clonal evolution occurring in practically every prostatic neoplasm.

Figure 6 illustrates the relationships between important kinetic properties of intraepithelial neoplasia. The rate of clonal evolution is defined as the rate of appearance in the neoplastic population of clonal variants that grow faster than surrounding cells (CVGF). The proliferation rate of neoplastic cells (NC) in the population determines the production rate of genomic structural variants (GSV), because each turn through the cell cycle converts damaged DNA lesions into mutations and also subjects the genome to a 10-fold greater mutagen sensitivity during S phase (45). A fraction of the GSV will be clonal variants that can grow faster (CVGF). Their focal expansions will add to the overall proliferation rate of NC. The final result is a continuously accelerating kinetic cycle involving increased production rates of NC, GSV, and CVGF, all of which are slowed by the increased production rate of apoptotic cells. The driving force of the cycle is entropic—that is, a selection pressure exists toward increasing disorder and heterogeneity as controls for maintaining homeostasis are lost.

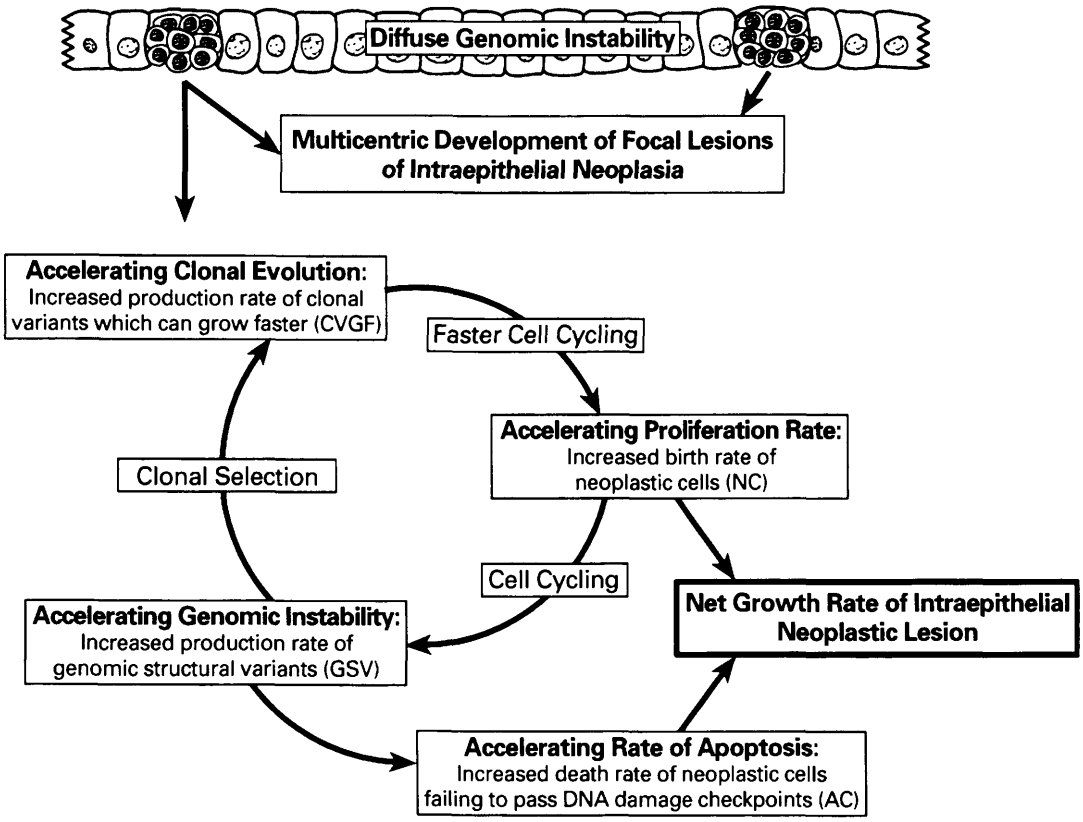


**Figure 6.** The continuously acclerating kinetic cycle of intraepithelial neoplasia. Proliferating intraepithelial neoplastic cells (NC) produce genomic structural variants (GSV), a fraction of which form clonal variants that grow faster (CVGF). CVGF add to the average proliferation rate, which increases the production rate of GSV. This in turn increases the production rate of CVGF, and the cycle repeats. This acclerating cycle is slowed by concurrent cell death due to increased production of apoptotic cells that have failed to pass DNA damage checkpoints. The difference between birth and death rates determines the net rate of growth in bulk of the intraepithelial neoplasm.

**Accelerating Intralesional Proliferation Rate.** The property of intraepithelial neoplastic lesions that their rates of clonal evolution and cell proliferation mutually augment each other is a variant of the famous dictum of Ames: "mitogenesis increases mutagenesis" (46).

**Accelerating Intralesional Apoptosis.** Figure 6 also shows the accelerating production of apoptotic cells (AC) that fail to pass DNA damage control checkpoints. The balance between neoplastic cell birth rate and death rate determines the net rate of growth in bulk of the intraepithelial neoplastic lesion.

The analysis illustrated in Figure 6 does take into account complexities such as (i) ischemic "oncosis" or anoxic death by swelling (47) stemming from inadequate capillary blood supply due to mechanical compression or insufficient angiogenesis factor production, (ii) augmentation of the intralesional cell proliferation rate by growth factors diffusing from adjacent hyperproliferating normal epithelium, or (iii) the stimulation of mitosis and apoptotic cell death induced by reactive oxygen species coming from inflammatory cell infiltrates. Nevertheless, a mechanistic rationale is provided to explain some important kinetic features of neoplasia, that the rates of both clonal evolution and intralesional cell proliferation tend to accelerate with time, and are slowed by an accelerating rate of apoptosis. Accelerating proliferation rates for cervical intraepithelial neoplasia have been demonstrated by Richart (48).

**Accelerating Intralesional Genomic Instability (Accelerating Production Rate of Genomic Structural Variants) prior to and during Intraepithelial Neoplasia, and after Progression to Invasive Carcinoma.**

*Prior to onset of intraepithelial neoplasia.* The finding of allelic loss of 9q in microscopically normal appearing hyperplastic epithelium adjacent to intraepithelial neoplastic lesions of the head and neck (49) is an example of a "predysplastic," or "premorphologic" change associated with genomic instability (Fig. 1). Another example is the demonstration by fluorescence-labeled *in situ* hybridization (FISH), using chromosome-specific centromeric probes, of multiple clones with aneusomy/polysomy occurring in normal, nonhyperplastic epithelium adjacent to intraepithelial neoplasia of the head and neck. The number and size of aneusomic clones increased with progression from normal nonhyperplastic to normal hyperplasia to dysplasia to cancer (50). As a third example, in sputum smears containing dysplastic cells as observed by conventional light microscopy there occur other cells which appear normal and nondysplastic, but when these same cells are observed by computer-assisted image analysis they show specific nuclear chromatin textural features that are seen only in smears containing dysplastic cells elsewhere. These predysplastic changes in the topographic distribution of DNA at the supramolecular level have been given the name of malignancy-associated changes, or MACs (51). On the other hand, in one report the normal epithelium adjacent to colorectal polyps showed no allelic losses (52).

*During intraepithelial neoplasia.* Vogelstein's original model of genetic progression during intraepithelial neoplasia of colorectal polyps illustrates the accelerating pace of genomic instability, as does a model Sidransky has developed of genetic progression in intraepithelial neoplasia of head and neck using microsatellite analysis of allelic loss. Sidransky's model shows that the earliest losses are of 9p in squamous hyperplasia described above, followed by losses of 3p and 17p in dysplasia, then of 11q, 13q, 14q in carcinoma *in situ*, and finally of 5p and 4q after invasion (49). In another example of progression of genomic instability in intraepithelial neoplasia, 61 cases of ductal carcinoma *in situ* (DCIS) of the breast exhibited a correlation between increased frequency of allelic loss (FAL) and an increase in nuclear grade (53). In a more detailed study, chromosomal losses of 16q and 17p occurred early in the genetic progression of DCIS, when it was low grade, followed by allelic losses of many more chromosome arms in lesions of intermediate and high grade (often including 1p, 1q, 6q, 11p, 11q, 13q, and 17q) (54).

*During invasive cancer.* Analyses in many laboratories of the allelotypes of many types of cancer have shown that the frequency of allelic loss, or FAL, (fraction of the 39 nonacrocentric autosomal chromosome arms showing structural loss), or if fewer loci are selected, the mean allelic loss, increases with time and tumor grade. A few recently published examples have been published for head and neck (55), urinary bladder (56), and prostate (57).

**Accelerating Phenotypic Heterogeneity.** The propensity of neoplastic populations to show an abnormal increase in phenotypic heterogeneity of structure and function is well established (58). Practically any geometric dimension, in particular nuclear area, shape, and chromatin texture, or any numerical count, such as the number of nucleoli or number of mitoses, have a mean and variance which both increase with time. The variance here is a quantitative measure of phenotypic heterogeneity. Accelerating phenotypic heterogeniety in an intraepithelial neoplastic lesion is the immediate consequence of the accelerating genotypic heterogeneity just discussed.

## Neoplastic Progression

The term neoplastic progression, illustrated for colorectal neoplasia in Figure 7, expands on the frequently expressed concept that carcinogenesis is a multistep process to encompass the complete continuum of evolving neoplastic change, from the first microscopic clonal expansion to the final terminal state of extensive bulk, invasiveness, disseminated metastasis, and death. Neoplastic progression may be defined as follows: The propensity of neoplasms over time to increase in total bulk and extent of dissemination (as defined by clinical stage) and to increase in the extent of deviation from normal cell and tissue structure and function (as defined by histopathological grade). The obvious clini-

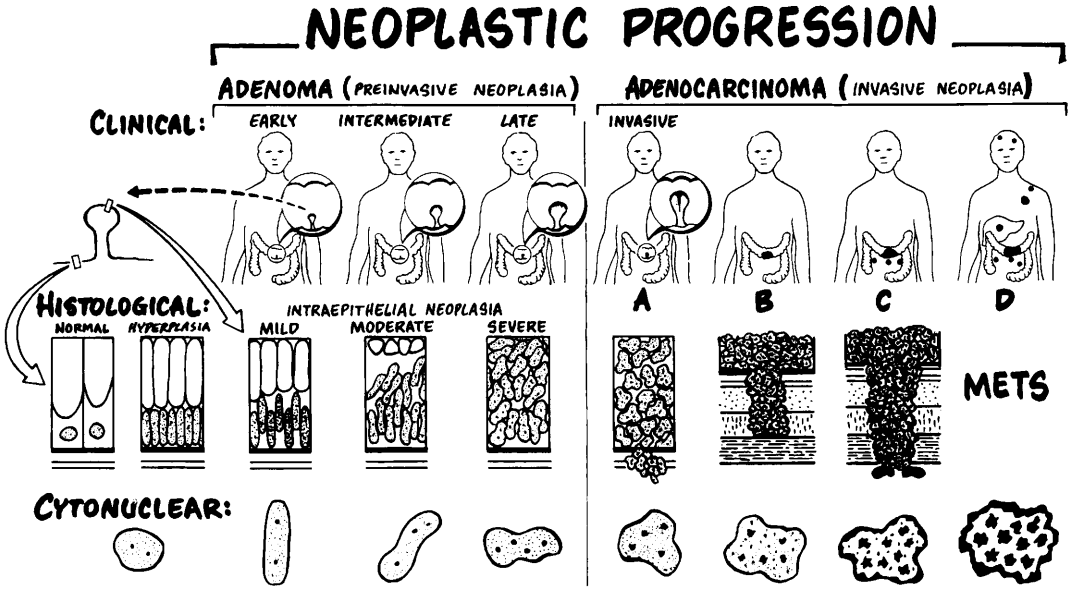


**Figure 7.** The complete span of neoplastic progression in colorectal neoplasia. The extent and rate of neoplastic progression may be diagnosed at either the clinical, tissue, or cytonuclear levels and used to estimate the probability of survival.

cal reality of neoplastic progression gives the simplest definition: neoplasms increase in Stage and Grade with time. Neoplastic progression has kinetic properties; the time-dependent succession of states of increasing bulk and variability of structure and function have both a rate and an extent. For a given cancer patient, measuring the extent of neoplastic progression provides an estimate of the survival time remaining to him. Figure 7 shows how the extent of neoplastic progression in colorectal neoplasia can be estimated from corresponding changes at three different levels of magnitude: the clinical level, the tissue level (extent of invasion and lymph node metastasis), and the cytonuclear level (extent of variation of nuclear size, shape, and especially chromatin texture). A fourth, molecular level may be added in the future, to include patterns of aneuploidy/aneusomy and other measures of the degree of genomic instability, such as extent of microsatellite instability and frequency of allelic loss. In the section on surrogate endpoint biomarkers below, it will be shown how chromatin texture patterns specific for neoplasia, quantitatively measured by computer-assisted image analysis, can be used to estimate the extent of neoplastic progression, and also to monitor slowing of the rate of neoplastic progression produced by chemopreventive agents.

**The Rate of Neoplastic Progression Is Driven by the Rate of Clonal Evolution.** From the above considerations, it is axiomatic that the characteristic clinical properties of neoplastic progression (viz, the constant increase in cell proliferative rates, in genotypic and phenotypic heterogeneity, and in total bulk and degree of dissemination) all derive from accelerating clonal evolution.

## Strategies of Chemoprevention Based on the Properties of Intraepithelial Neoplasia

Recognizing that intraepithelial neoplasia is a condition in which multiple neoplastic foci develop in an epithelium affected with genomic instability, and that the foci enlarge slowly over many years, the prime strategy of chemoprevention is to diagnose and treat intraepithelial neoplasia as early as possible, even during the predysplastic phase of diffuse genomic instability, with agents that maximally slow neoplastic progression, or, of course, abrogate it altogether. Chemopreventive agents under development by the chemoprevention program at the National Cancer Institute have been reviewed extensively (59–61).

**Emphasis on Early Diagnosis and Therapy of Intraepithelial Neoplasia.** FISH assays are now available to diagnose and monitor the treatment of diffuse genomic instability, even before the onset of intraepithelial neoplasia in patients with proven high cancer risk (e.g., previous surgery for head and neck cancer). Diagnostic assays for microsatellite instability in urine (62), and sputum (63), and *ras* gene mutations in stool (64) have been developed. In a later section, computerized imaging methods will be described for diagnosing and monitoring treatment of neoplasia-specific changes in cytonuclear chromatin texture during early intraepithelial neoplasia. At the clinical level, a recent advance in early diagnosis is the LIFE system of fluorescent bronchoscopy (65) which induces natural autofluorescence in intraepithelial neoplastic lesions of the pulmonary bronchi so that they can be easily located for biopsy and analysis.

**Emphasis on Developing Antiproliferative Agents.** The central and powerful role played by hyperproliferation in driving the accelerating rate of progression of intraepithelial neoplastic lesions is undeniable, and justifies aggressive development of new and more powerful antiproliferative agents. Drugs need development which inhibit steps in signal pathways related to initiation of cell proliferation (e.g., growth factors and their receptors, and the *ras*/*raf*/MAP kinase pathways) and steps in synthesis

pathways duplicating cell structure in preparation for cell division (e.g., enzyme-catalyzed steps in deoxyribonucleotide synthesis). Agents that are strictly proliferation suppressants, such as difluoromethylornithine, are cancer preventive in diverse animal models in the chemoprevention program (66). Since proliferative rates of advanced grades of intraepithelial neoplasia are generally 5- to 10-fold higher than those of normal epithelia, proliferation suppressants may tend to have relatively greater effect on neoplastic tissue. Interfering early enough during the development of intraepithelial neoplasia with proliferation-suppressant therapy has the potential of adding a much more extended period of wellness and reduced cancer risk.

**Emphasis on Developing Anti-Inflammatory Agents.** Intervention with nonsteroidal anti-inflammatory agents should be considered in every subject whose intraepithelial neoplasia is likely to be associated with chronic inflammation, for example, lesions of the respiratory and digestive tracts and the uterine cervix. There are three enzyme activities associated with inflammation whose inhibition should be considered: the prostaglandin endoperoxide synthase and hydroperoxidase activities which generate prostaglandins, and the lipoxygenase activity which generates leukotrienes. The hydroperoxidase activity is important because it will "co-oxidize" and activate carcinogens (67). Agents that inhibit both prostaglandin endoperoxide synthase and the lipoxygenase, such as the plant phenolics curcumin and quercetin, appear to be of advantage.

**Emphasis on Developing Antioxidants.** Reactive oxygen species (singlet oxygen, superoxide, peroxide, hydroxy free radicals) and related endogenous free radicals (peroxynitrite, nitric oxide, hypochlorite) are both mutagenic and mitogenic (68). They are of common occurrence in the environment, particularly in cigarette smoke and fossil fuel combustion products, and are endogenously produced extracellularly by chronic inflammatory infiltrates and intracellularly by "leaky flavoproteins" in the electron transport chain of mitochondria and in cytochrome P450 reductase of the endoplasmic reticulum (69). Antioxidants appear to offer good potential for general chemopreventive action. Plant phenolics such as curcumin and flavonoids, in particular, have both antioxidant action by blocking lipid peroxidation, and anti-inflammatory action by blocking the enzyme lipoxygenase (70) which produces leukotrienes that are chemotactic attractants to inflammatory cells.

**Antimutagenic Agents.** Preventing the mutagenic effects of chronic tobacco use is a common indication for intervention with antimutagenic agents. Antimutagens with different primary mechanisms of action are being tested—for instance, Oltipraz, which induces Phase II xenobiotic metabolizing enzymes (glutathione synthetase, glutathione-*S*-transferase, epoxide hydrolase, UDP-glucuronyl transferase), and alkylaryl isothiocyanates, which suppress Phase I oxidizing enzymes (arylhydrocarbon hydroxylases). Since the availability of *l*-cysteine is rate-limiting to the activities of glutathione synthetase, gluathione-*S*-transferase, and glutathione peroxidase, antimutagens that induce these enzymes should be given in combination with *N*-acetylcysteine, which provides *l*-cysteine immediately on being transported into cells.

**Emphasis on Developing Proapoptotic Agents.** When a proapoptotic agent induces a rate of apoptosis that exceeds the rate of proliferation, it will cause the neoplasm to shrink and disappear. For example, sulindac causes apoptosis and shrinkage of established colonic polyps in patients with familial adenomatous polyposis (71), and the terpene perillyl alcohol produces massive apoptosis in established mammary tumors of rats, causing the tumors to disappear completely (72) (confirmed by the senior author, who examined histological slides provided by Dr. Michael Gould). Animal models to screen for proapoptotic agents should be set up with protocols for late intervention that test for shrinkage of already established tumors.

**Preventing "Clonal Escape" from the Effects of Chemopreventive Agents.** Considering that intraepithelial neoplasia is characterized by multicentrically distributed and independently progressing neoplastic lesions undergoing clonal evolution, when a chemopreventive agent is adminstered, the risk increases with time that clonal variants will arise that have switched to metabolic pathways other than those being affected by the chemopreventive agent, so that "clonal escape" ensues. The best chemopreventive strategy to combat clonal escape is to use combinations of chemopreventive agents with different mechanisms of action.

## Development of Surrogate End Point Biomarkers for Chemoprevention Trials

**Need for Surrogate End Point Biomarkers.** Cancer chemoprevention trials that use the end point of cancer incidence reduction are faced with two frustrating problems. First is the long time (many years, even decades) required to follow intraepithelial neoplastic progression to the point of invasion, and second is the large number (many thousands) of subjects usually required to reach statistical significance from the survival data. Surrogate (i.e., substitute) end point biomarkers (SEBs) are being actively sought which will solve these two problems. The aims are, first, to measure events that occur much earlier in the progression of intraepithelial neoplasia, but still correlate highly with the ultimate outcome of invasive cancer, and, second, to use assays of such accuracy and precision (low variance) that adequate study power can be achieved with fewer patients. The various classes of possible SEBs have been critically reviewed previously (73). Briefly, these classes are related to abnormally high proliferation rate, genomic instability (including aneuploidy), nuclear/nucleolar structural abnormalities measured by computer-assisted quantitative image analysis (CQIA), oncogene activation and tumor suppressor gene inactivation, differentiation molecules (including fibers, adhesion molecules, and glycoconjugates), growth factors and their receptors, and various metabolite levels.

**Use of Computer Assisted Quantitative Image Analysis To Measure SEB.** In present-day histopathologic diagnosis, the established practice of subjectively estimating cancer risk by assessing nuclear grade, using relatively imprecise descriptive terms such as nuclear pleomorphism and hyperchromasia or moderately increased number of mitoses, will no doubt continue for the indefinite future. Nevertheless, computer-assisted quantitative image analysis (CQIA) is being used increasingly to measure the morphometric and DNA densitometric parameters of intraepithelial neoplasia and may one day come into general use.

Basically, two modalities are used in CQIA. One, cytomorphometry, measures geometric relationships, such as nuclear dimensions and nucleolar size, shape, and position. The other, cytophotometry, measures nuclear DNA optical density (DNA ploidy) and chromatin texture. The range of applications of image cytophotometry is wide because it also can be used to quantify the intensity and location in tissue sections of specific antibody or cDNA probes conjugated to a chromogen-generating molecule (e.g., a fluorescent dye or horseradish peroxidase).

**Selection of the Most Useful SEBs.** In searching for some property of early intraepithelial neoplastic progression that correlates with high cancer risk, the morphological nuclear changes of intraepithelial neoplasia immediately suggest themselves, particularly if they are to be measured quantitatively and objectively by means of CQIA. Such nuclear morphology-based SEBs have been critically reviewed previously (74). Briefly, they are increased nuclear size, altered nuclear shape, increased variance of nuclear size and shape (pleomorphism), altered chromatin texture, increased mitotic index, abnormal mitoses, and alteration or absence of differentiation and maturation. These SEBs have the advantage that they are not simply markers of intraepithelial neoplasia; they *are* intraepithelial neoplasia, by definition. Measuring morphonuclear changes and proliferative behavior of intraepithelial neoplastic lesions as predictors of later invasive neoplasia may be confounded by the fact that spontaneous regression of some of these lesions may occur, especially if the lesions are mild to moderate in extent. Therefore, in addition to quantitatively evaluating intraepithelial neoplastic lesions in biopsy samples from the same patient before and after intervention with a chemopreventive agent, comparison should also be made with lesions in control subjects given placebos.

The core end points now being used in over 80 chemoprevention clinical trials include morphonuclear SEBs (nuclear and nucleolar size, shape, variance of size and shape, frequency of number of nucleoli per 100 cells, and particularly dozens of chromatin texture features), DNA ploidy, and proliferative index measured with antibody probes, all quatitated objectively with CQIA.

**Design of Computer Software To Detect Nuclear Chromatin Texture Features Specific For Neoplastic Changes.** Dozens of nuclear chromatin texture features have been measured in neoplastic cells, based on analysis of patterns of change in optical density from one small pixel, 0.5 $\mu$m $\times$ 0.5 $\mu$m in size, to another in the digitized image of the nucleus. A new computer instrument with new software programs developed by Bacus Laboratories Imaging Systems, called the BLISS instrument, measures quantitatively and objectively many of the same nuclear chromatin texture features used by histopathologists to make a diagnosis of intraepithelial neoplasia. An example of the measurement of one chromatin texture feature will be presented here, using the Deep Valley Detector.

**The Deep Valley Detector, a Software Program That Detects Nuclear Features Specific for Neoplasia.** The cell nuclei of intraepithelial neoplastic lesions are commonly described by the pathologist as showing chromatin clumping. The edges of the chromatin clumps are said to be sharply marginated. John Frost, the late Chief of Cytopathology at Johns Hopkins, coined the term ''cookie-cutter chromatin'' to describe this characteristic sharp margination. He also emphasized that the space between chromatin clumps is lighter than normal, and called this space, as others have, parachromatin clearing. Figure 8 compares the nuclear chromatin pattern in a normal hyperplastic cell

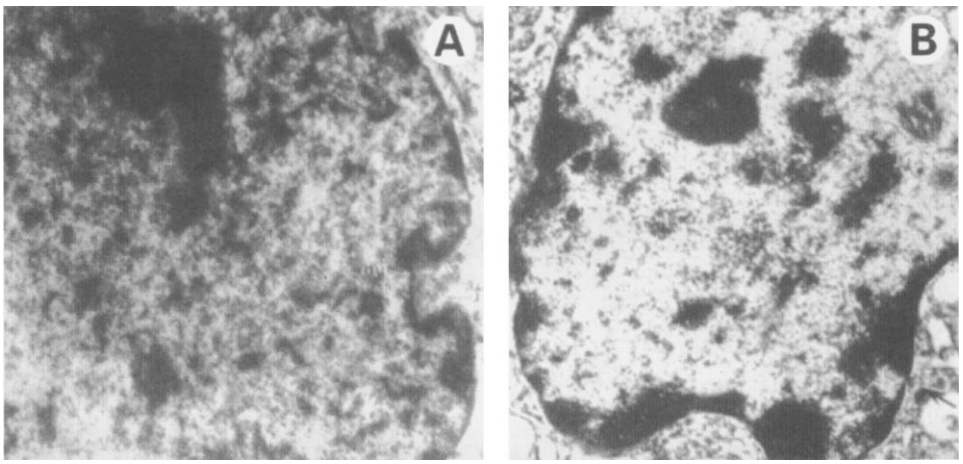


**Figure 8.** Low-power electron micrographs of cell nuclei from the uterine cervix. (A) Nucleus of a hyperplastic normal cell. (B) Nucleus of an intraepithelial neoplastic cell of high grade. Note the dense chromatin clumps with sharp margins bordering intervening regions that stain more lightly (so-called parachromatin clearing).

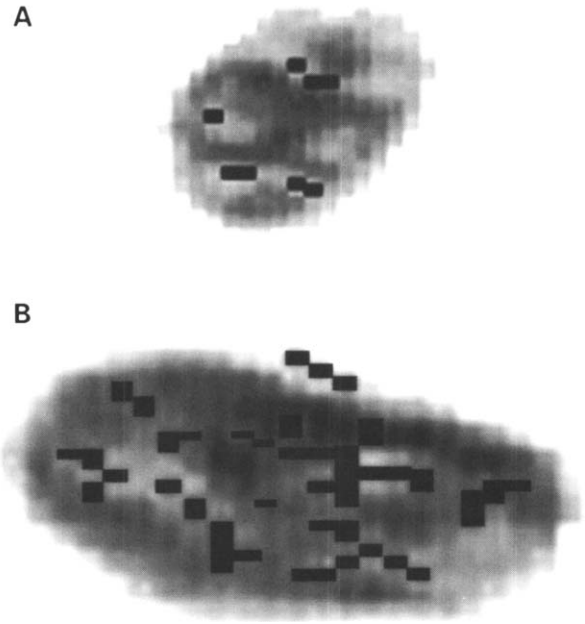


**Figure 9.** Representative images of cell nuclei from the uterine cervix displayed by the computer showing the location of deep valley sites. (A) Nucleus of a normal hyperplastic cell, with 16 sites. (B) Nucleus of a neoplastic cell, with 111 deep valley sites.

of the uterine cervix with that of a neoplastic cell from the same tissue. The sharply marginated chromatin clumps and lighter area between the clumps of the neoplastic nucleus can easily be seen. A software program was designed that directs the computer to identify the number and location within the nucleus of a specific pixel pattern associated with sharp-edged chromatin clumps adjacent to areas of parachromatin clearing. The following command was given to the computer: "Find every set of three pixels in a row, the center pixel of which has an optical density which is less than the optical density of either end pixel by at least 0.05 OD units. Count the number of pixel triplets with this property and mark their location in an image of the nucleus." This program is called the Deep Valley Detector, because it identifies the margins of chromatin clumps in neoplastic nuclei that have a steep and deep optical density dropoff. The depth of the dropoff which is detected can be varied. Figure 9B shows the computer image of a nucleus from a neoplastic cell of high-grade cervical intraepithelial neoplasia, in which 111 "deep valley" sites were counted and their location shown (by a red mark in the original image). By contrast, Figure 9A is the nucleus of a non-neoplastic hyperplastic cell from adjacent cervical epithelium, which had only 16 sites. This large quantitative difference in number of deep valley sites between normal hyperplastic and neoplastic nuclei was generally found for all nuclei in the specimen. The number of deep valley sites, including those of different depths, may be used to measure with precision the extent of neoplastic progression and also the modulating effects of chemopreventive agents on intraepithelial neoplasia.

## Summary

A central goal of the chemoprevention program is to learn as much as possible about the molecular and cellular mechanisms of intraepithelial neoplasia, and to use this information to plan directions with the best chance of success. Intraepithelial neoplasia has two precursor conditions and four major properties. The first precursor condition, chronic hyperproliferation, in common but not essential. The second, genomic instability, is essential. It is defined as an increased rate of unrepaired DNA breaks with secondary formation of abnormal genomic structural variations, including oligonucleotide mutations, allelic loss and gain, and karyotypic (whole chromosomal) aberrations in structure and number. The four major properties of intraepithelial neoplastic lesions relevant to chemoprevention are (i) multifocality, (ii) clonal evolution, (iii) accelerating intralesional production of genomic structural variants cells (some of which form clones that grow faster and others which undergo apoptosis due to recognition by checkpoint controls of excessive DNA damage), and (iv) increasing phenotypic heterogeneity. Efficient planning strategies for a chemoprevention program include the following emphasis: (i) early diagnosis and therapy, (ii) development of more agents in the categories of antiproliferatives, antioxidants, antiinflammatories, and proapoptotics, (iii) the prevention of clonal escape by using combinations of chemopreventive agents, and (iv) the use of computer-assisted quantitative analysis to evaluate modulation of cell and tissue surrogate end point biomarkers in chemoprevention clinical trials.

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