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# The Natural History of Intraepithelial Neoplasia: Relevance to the Search for Intermediate Endpoint Biomarkers

Charles W. Boone, Gary J. Kelloff, and Vernon E. Steele

Chemoprevention Branch, Division of Cancer Prevention and Control, National Cancer Institute, NIH, Bethesda, Maryland 20892

**Abstract** The development of carcinomas, defined as invasive epithelial neoplasms, is preceded by a preinvasive stage termed intraepithelial neoplasia that typically lasts for years. Intraepithelial neoplasia is the target tissue for the action of chemopreventive agents and the site where biomarkers frequently develop. The term "dysplasia" refers to the morphological alterations that characterize intraepithelial neoplasia and, according to many authors, consists of seven basic changes that are the same for the majority of epithelia. These are increased nuclear size, abnormal nuclear shape, increased nuclear stain uptake, nuclear pleomorphism (increased variation in size, shape, and stain uptake), increased mitoses, abnormal mitoses, and disordered or absent differentiation. Clonal evolution appears to begin early in the neoplastic process during intraepithelial neoplasia. The use of intraepithelial neoplasia as an intermediate endpoint biomarker requires that effective chemopreventive agents cause it to regress. Two examples are the regression of dysplastic oral leukoplakia produced by beta-carotene and the regression of colonic polyps in familial polyposis patients following treatment with the nonsteroidal antiinflammatory drug sulindac. There is a critical need to identify and develop biomarkers that correlate with the appearance and regression of intraepithelial neoplasia. 

**Key words:** biomarkers, chemoprevention, clonal evolution, dysplasia, genomic instability, intermediate biomarker, intermediate endpoint biomarker, intraepithelial neoplasia, precancerous

Carcinomas, defined as invasive epithelial neoplasms, are typically preceded by a preinvasive stage termed intraepithelial neoplasia that may last for years. Intraepithelial neoplasia is important to study in relation to biomarker research because it is frequently the tissue site where biomarkers develop. It is also a target tissue for the action of chemopreventive agents intended to slow or stop its development.

Intraepithelial neoplasia typically begins as a monoclonal [1] focus of morphologically altered stem cells next to the basement membrane that expands upward and laterally until the full thickness of the epithelium is involved, after which it continues to spread laterally. The term "dysplasia" is conventionally applied to the collection of changes in cellular morphology and differentiation pattern that define the presence of neoplasia within the epithelium. When the intraepithelial neoplastic cells finally invade across the basement membrane, the neoplastic lesion is by definition now called "malignant," "carcinoma," or "cancer," and the morphological changes called "dysplasia" before invasion are now called "anaplasia."



A complete review of the natural history of intraepithelial neoplasia in humans, with implications for cancer chemoprevention strategy, has recently been published [2].

## MORPHOLOGIC CHARACTERISTICS OF INTRAEPITHELIAL NEOPLASIA

From a survey of the literature there appear to be seven basic morphological criteria included in the term "dysplasia" that define the presence of intraepithelial neoplasia. These are: 1) increased nuclear size; 2) abnormal nuclear shape; 3) increased nuclear stain uptake; 4) nuclear pleomorphism (abnormal variation in size, shape, and stain uptake); 5) increased mitoses; 6) abnormal mitoses; and 7) disordered or absent differentiation. All of these criteria have been specifically included in the term "dysplasia" in articles on uterine cervix [3–6], oral leukoplakia

[7,8], larynx [9], lung [10], esophagus [11], colon [12–14], urinary bladder [15–17], and skin [18].

The severity of intraepithelial neoplasia is estimated from the extent of the lesion as well as the degree of deviation from normal cellular morphology and differentiation pattern. As the initial clonal focus of neoplastic cells near the basement membrane (referred to above) expands upward and laterally within the epithelium, it is called "mild" dysplasia when limited to the lower third of the epithelium; "moderate" dysplasia when limited to the lower two thirds; and "severe" dysplasia when it occupies the full thickness of the epithelium. The term "carcinoma *in situ*" is also used for "severe dysplasia," but there is much well-reviewed evidence that severe dysplasia and carcinoma *in situ* cannot be reliably distinguished [19]. "Carcinoma *in situ*" is the less preferred term because it has misled some into assuming that the neoplastic process actually starts at this point, and that the changes of dysplasia which precede it are "preneoplastic." On the contrary, as emphasized by Leslie Foulds [20], the neoplastic process is a single continuum from the initial monoclonal focus of dysplastic cells near the basement membrane to the large and full-thickness lesion of severe dysplasia that invades across the basement membrane to become an enlarging and disseminating carcinoma.

## CLONAL EVOLUTION DURING THE DEVELOPMENT OF INTRAEPITHELIAL NEOPLASIA

The process of clonal evolution was first documented in hematopoietic and lymphopoietic neoplasms by Nowell, and is generally considered to be present in tumors in general [21,22]. Clonal evolution is defined as the continuous production within a tumor cell population of genetically variant cells, with selection and clonal expansion of those variants that have an additional growth advantage under the prevailing set of selection pressures. Genetic instability, manifested by gene mutations, gene amplifications, chromosomal structural changes and aneuploidy, is postulated to be the basis for the increased production of genetically variant cells associated with clonal evolution. Of these genetic changes, aneuploidy is the easiest to determine because of recent advances in flow and image cytospectrophotometry.

It is now apparent that clonal evolution also takes place during intraepithelial neoplasia. A clear example, as described by Vogelstein *et al.* [23], is seen in colorectal adenomatous polyps, the epithelium of which exhibits intraepithelial neoplasia. The progression from early to late colorectal adenomas is associated with activation of the *ras* oncogene by mutation and inactivation of various tumor suppressor genes [23]. Each alteration confers a growth advantage that results in a wave of clonal expansion.

Aneuploidy has been documented by flow cytometry in a large percentage of cases of severe dysplasia of cervix [24], skin [25], oral leukoplakia [26], larynx [27], lung [28], esophagus [29], stomach [30], and colorectum [31]. Flow cytometry cannot detect less than a 5% increase of total DNA, so that aneuploidy involving two or three chromosomes may not be detected [32]. In a comparative study, colonic polyps that were 20% aneuploid by flow cytometry were 80% aneuploid by karyotypic analysis. In this study, even polyps with mild dysplasia were aneuploid [33].

Since each sequentially expanding clone possesses a growth advantage conferred by a genomic alteration, the repeated waves of clonal expansion should be associated with a general increase in mitotic frequency of the intraepithelial neoplastic cell population. This has been confirmed in a study of cervical intraepithelial neoplasia, where the tritiated thymidine labelling index rose from a normal of 4.6% to 10.3% during mild dysplasia, and to 46.5% during "carcinoma *in situ*" [34].

## THE USE OF INTRAEPITHELIAL NEOPLASIA AS AN INTERMEDIATE ENDPOINT BIOMARKER

The two minimum requirements of an intermediate endpoint biomarker are: 1) that it must correlate with cancer risk, and 2) that it must be modulatable toward normal by an effective chemopreventive agent. In considering whether intraepithelial neoplasia can be used as an intermediate endpoint biomarker, the question becomes, will severe dysplasia, particularly when associated with aneuploidy, regress toward normal when acted on by an appropriate chemopreventive agent? There are three published examples of a chemopreventive agent

causing regression of intraepithelial neoplasia. One is by Garewal [35], who presents photographs of histologic sections of a leukoplakic lesion of the oral epithelium in a patient before and after a three month course of oral β-carotene. The initial biopsy showed moderate dysplasia with multiple abnormal mitoses, indicating that aneuploidy was present. After the patient had received oral β-carotene, the lesion appeared to regress macroscopically, and a second biopsy at the site of the lesion revealed only normal oral mucosa. The second example is by Hong *et al.* [36] who describe the regression of dysplastic oral leukoplakic lesions, including severe dysplasia to normal epithelium, produced by three months of oral 13-*cis*-retinoic acid. The third example, reported by several authors [37,38], is the regression of colorectal polyps induced by sulindac (nonsteroidal antiinflammatory) in patients with familial polyposis and Gardner's syndrome. The most recent report [39] describes a randomized, placebo-controlled, double blind, crossover study in 10 patients with familial polyposis who had residual rectal polyps after colectomy and iliorectal anastomosis. Oral sulindac induced complete regression of polyps in 6 patients and almost complete regression in 3 patients. After discontinuance of sulindac therapy, recurrence of polyps occurred in some, but not all, patients within 3–4 months. A second course of sulindac produced complete regression of the recurrent polyps.

Although the above examples of induction of regression of intraepithelial neoplasia by a chemopreventive agent need confirmation and elucidation of mechanism, they do demonstrate the possibility of using intraepithelial neoplasia as an intermediate endpoint biomarker. It appears likely that more examples in different tissues will appear as new chemopreventive agents with a regressive effect on intraepithelial neoplasia continue to be identified.

The development of intraepithelial neoplasia is associated with the appearance of a number of individual biomarkers that have been categorized as genomic (oncogene activation, gene amplification, abnormal structure and number of chromosomes), proliferative [thymidine labeling index, nuclear antigens such as Ki-67 and proliferating cell nuclear antigen (PCNA)], and differentiation related (abnormal glycoconjugate antigens, loss of cytoskeleton antigens). Since the regression of intraepithelial neoplasia should correlate with changes in these associated biomarkers, it would be desirable if one, or preferably a battery, of the biomarkers could be developed to provide a more objective and quantitative measure of regression of intraepithelial neoplasia than the present method based on the evaluation of the morphological changes of dysplasia by a specialist. More importantly, these biomarkers are urgently needed for use as intermediate endpoints in clinical trials of chemopreventive agents. Their use would supplant the presently used endpoint of cancer incidence reduction, which requires large study populations, long observation periods, and great expense. For this reason, the development of intermediate endpoint biomarkers that correlate with the appearance of intraepithelial neoplasia should be vigorously pursued.

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