Chromosomal Deletions and Progression of Premalignant Lesions: Less Is More

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Epithelial malignancies arise after a series of progressive pathologic changes including hyperplasia, different grades of dysplasia, and carcinoma *in situ* (1). These premalignant changes can accompany cancer or may occur in the epithelium of individuals at high risk. Sequential premalignant lesions have been defined for many epithelial tumors, including oral squamous cell carcinoma (1) and Barrett's esophageal adenocarcinoma (3). Two of the most exciting areas of current cancer research are (a) novel high-throughput technology for molecular studies of carcinogenesis and (b) the use of this technology in discovering and characterizing genetic abnormalities that underlie the progression of epithelial premalignancy.

Many studies of the last 2 decades have helped to characterize molecular changes of epithelial premalignancy. Encouraged by the development of methodologies (such as laser microdissection) for isolating cells from small histologic lesions, and of techniques to do genomic studies on minute amounts of DNA, several groups have made substantial progress in unveiling the molecular and genetic abnormalities of epithelial premalignant lesions (2, 3–7). These changes involve inactivation of known and putative tumor suppressor genes and several dominant oncogenes. Tumor suppressor genes are believed to be inactivated via a two-step process involving both alleles. Knudson (8) proposed that the first "hit" frequently is a point mutation, whereas the second allele is subsequently inactivated via a chromosomal deletion, translocation, or other event such as methylation of gene promoter regions (9). Dominant oncogenes are frequently activated by mutation, increased copy number, and translocations (9).

The general working model of sequential molecular abnormalities in the pathogenesis of epithelial tumors indicates that genetic changes (*a*) commence in histologically normal epithelium and progress with the increasing severity of epithelial changes, (*b*) follow a sequence from early to late changes, and (*c*) are extensive and multifocal throughout the epithelium, indicating a field effect, also known as "field cancerization" (3). Therefore, in various organ sites, multiple clonal and subclonal patches of molecularly abnormal epithelial cells (with or without cytologic and morphologic abnormalities) can be detected throughout the affected epithelium. New high-throughput genomic and proteomic profiling techniques are now starting to be applied to premalignant or normal-appearing tissue because these techniques are suitable for studying the small amounts of tissue usually available in these precancer settings.

Oral squamous cell carcinoma and Barrett's esophageal adenocarcinoma are on the frontier of innovative discoveries involving molecular events in the progression of premalignant lesions (2, 3). These two diseases are good models of cancer genetic progression because the premalignant epithelium can be safely visualized and biopsied so that genomic changes can be compared in different stages of neoplastic evolution and then studied longitudinally by biopsy surveillance. Several studies have shown that the general working model of progression described above applies to both tumor types.

Oral leukoplakia is the most common head and neck premalignant lesion and has a malignant transformation rate as high as 24% (10). Deletion of one of the two alleles within chromosome arm 3p and chromosome segment 9p21 is the most frequent event in oral lesions with only mild dysplasia and even in some normal-appearing epithelial cells (11). Oral leukoplakias with deletions in the 3p14 (*FHIT*) and 9p21 (*CDKN2A*) regions are reported to carry a higher risk for transformation (2). Barrett's esophagus (BE) is the only known premalignancy of esophageal adenocarcinoma; only a small fraction (0.5-1% annually) of BE patients subsequently develop adenocarcinoma (12). Although less accessible than are oral lesions, BE is a unique model for the study of human neoplastic progression *in vivo*. The standard care of BE requires biopsies according to defined protocols at multiple time points from the same patient, allowing the generation of spatial maps and longitudinal evaluation of genetic alterations that arise during clonal evolution. Several studies of BE indicate that inactivation of *CDKN2A* by loss of heterozygosity (LOH), methylation, and/or mutation is selected as an early event that predisposes to large clonal expansions of the BE tissue (12, 13). Subsequent inactivation of *TP53* by mutation and LOH predisposes to progression to aneuploidy and invasive adenocarcinoma development (13). All these studies have been done using low-throughput methodologies with analysis of abnormalities on few chromosomal foci or specific genes.

In the present issue of the journal, Tsui et al. (14) and Li et al. (15) report their high-throughput analyses of genetic abnormalities in premalignant lesions that provide insights on chromosomal changes in the early pathogenesis of oral squamous cell carcinoma (14) and BE (15). These studies are among the first to use high-throughput DNA copy number analysis by microarrays in the study of the sequential progression of premalignant lesions of any organ site. Comparative genomic

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hybridization (CGH) profiling has been previously applied to oral squamous cell carcinoma (16–18), leading to the identification of novel chromosomal regions (e.g., 5p15.2 and 11q.22.2-22.3) frequently altered in this neoplasm (16). Earlier studies using GCH and single-nucleotide polymorphism (SNP) array have already shown that chromosomal abnormalities increase with the progression of BE lesions, but they analyzed only a few tissue specimens (19, 20). Both of the present studies showed that microarray-based DNA copy number analyses can reveal the progression of chromosomal abnormalities that parallels the clinical and histopathologic progression of premalignancy. The findings of these studies also have some potential to be used to predict the risk of progression of either oral leukoplakia or BE.

Tsui et al. (14) used CGH to evaluate genetic alterations on chromosome 3p in 47 oral premalignant lesions with high-grade dysplastic features (severe dysplasia and carcinoma *in situ*) and compared these alterations with findings in 23 oral squamous cell carcinomas; all samples were formalin-fixed and paraffin-embedded tissues. This study was stimulated by previous laboratory work of this group (21, 22) and others (2, 10, 11), showing that chromosome 3p is frequently altered in the early pathogenesis of oral carcinoma and has been associated with the risk of premalignant lesion progression (2, 10, 11, 21, 22). High-grade dysplastic lesions exhibited six recurrent regions of losses on 3p including 3p25.3-p26.1, 3p25.1-p25.3, 3p24.1, 3p21.31-p22.3, 3p14.2, and 3p14.1, which overlapped losses found in invasive carcinoma. Next, the authors examined these regions in 24 low-grade dysplastic lesions with known outcomes, including 2 hyperplasias and 22 mild and moderate dysplasias, and determined that 3p losses were identified more frequently in low-grade dysplastic lesions with progressive behavior (78%) compared with nonprogressing lesions (20%). This interesting observation obviously will require verification in a larger cohort of patients. In our opinion, one remarkable finding of this report is that the size of 3p segmental losses, or discontinuous LOH (alternating segments showing loss and retention; portrayed in Fig. 1), increased with histologic stage; segmental 3p losses were detected in premalignant lesions, whereas whole-arm loss occurred mainly in invasive tumors.

Li et al. (15) used a medium-density SNP array (containing ~33,000 SNPs) to investigate genome-wide chromosomal copy number changes in whole frozen tissue specimens (obtained by endoscopy or surgical resection) of multiple stages of BE and esophageal adenocarcinoma from 42 patients. This investigation is from the same laboratory that established the working model for BE molecular progression (3) and one of the first laboratories to use SNP arrays for the analysis of human premalignancy (23). The mucosal esophageal tissues used in this cross-sectional study included 24 early-stage BE specimens (with or without aneuploidy and that did not develop cancer during follow-up), 10 late-stage BE specimens (with microscopic invasion), and 8 grossly invasive esophageal adenocarcinoma specimens. Genome-wide copy losses and LOH (the most frequent changes) and copy gains increased in frequency and size between early and late BE, with SNP abnormalities increasing from 2% in early stages to >30% in late stages. A set of statistically significant events was unique to either early or late BE stage, and few chromosomal regions with changes were common in all stages of progression. It is interesting that the total number of genome-wide SNP alterations (gains, losses, and LOH) was highly correlated with DNA content aneuploidy and was sensitive and specific for identifying patients with concurrent esophageal adenocarcinoma. As with chromosome 3p in the study of Tsui et al. (14), Li et al. found that the sizes of chromosome abnormalities were small in early-stage BE compared with late stages, including invasive adenocarcinoma, except in the case of 9p LOH.

How do the articles by Tsui et al. (14) and Li et al. (15) contribute to the general concepts of the parallel progressions of genetic and clinical/histopathologic changes in premalignancy (described above)? First, these investigators have successfully applied high-throughput DNA chromosomal abnormality analysis technologies, array CGH and SNP array analysis, to small specimens of premalignant lesion tissue, formalin-fixed and paraffin-embedded specimens (CGH), and frozen samples (SNP). We hope that these two reports will raise enthusiasm for similar new studies in these and other neoplasm models.

Second, these studies show that chromosomal abnormalities (mostly deletions) at the 3p chromosome (14) and genome-wide (15) levels commence early in oral and BE premalignancies and progress with the increasing severity of epithelial changes and that these chromosomal changes follow a sequence defining early and late molecular changes. Third, these two studies have suggested that the size of chromosomal deletions (at 3p or genome-wide) increases with the severity of histopathologic changes. This interesting finding has been reported previously in the pathogenesis of other epithelial tumors, such as lung tumors, where it has been observed in LOH studies using PCR-based amplification of multiple microsatellites in precisely microdissected histologically normal and premalignant epithelia obtained from lung cancer patients and high-risk individuals (smokers; refs. 24–26). LOH at multiple chromosome 3p (24, 26) and 8p (25) sites was shown to commence at the stage of normal epithelium and to increase with progressive histologic changes in the lung squamous cell carcinoma histology progression model (similar in ways to oral squamous carcinoma). This study used 24 microsatellite markers spanning six continuous chromosome 3p regions in showing that deletions in 3p progressed from dysplasia to invasive squamous carcinoma (Fig. 1). In invasive tumors and carcinoma *in situ*, most of the 3p arm was deleted and the extent of the deletions was greater in all cases than that in corresponding normal and premalignant foci (24). These findings were subsequently expanded in a LOH analysis (using 54 microsatellite markers on 3p) that included samples from a wider spectrum of premalignancy and lung cancer specimens, including samples from smokers and cancers with different histologies (nonsmall and small cell). This detailed allelotyping analysis identified multiple areas of discontinuous LOH and thus multiple breakpoints throughout the 3p arm in many tumor and bronchial epithelial samples (26). Allelic losses present in lung premalignant lesions were not random and followed a sequence, with the earliest and most frequent allelic loss occurring at the 3p21.3 region (24, 26).

Although the two articles in this issue of the journal report that discontinuous LOH marks progression of premalignancy, this LOH also could be an artifact of impurity within the premalignant lesions. In samples with an admixture of DNA from malignant and normal cells, some chromosomal segments

might seem to be altered and others not altered, although the degree of alteration could be the same across the entire chromosome arm because of nonuniform behavior of copy number probes, which fail to reach a threshold level for detecting an alteration. This failure has been shown for SNP array analysis of mixtures of tumor cell line DNA with matched normal DNA; increasing admixture of normal DNA led to the appearance of discontinuous LOH because of incomplete detection involving regions of uninterrupted chromosome 3p LOH in the pure tumor (27). Further improvements in genomics technologies coupled with careful histologic analysis will be required to address this question.

The mechanism for chromosomal deletions in individual premalignant samples is not well established. LOH is considered to occur through the loss of a whole chromosome because of inappropriate chromosomal segregation at mitosis and also through genetic alterations that change chromosomal structures (28). Whole, terminal, and interstitial chromosome physical deletions have been shown to cause LOH on several chromosomal arms in human tumors (29). On the other hand, mitotic recombination and gene conversion seem to be additional mechanisms causing LOH (30, 31). Unbalanced translocations, which have been confirmed by cytogenetic studies in certain human tumors, have been implicated in LOH development (32). In summary, six chromosomal aberrations (whole chromosome, terminal and interstitial deletions, mitotic recombination, gene conversion, and unbalanced translocation) are considered to be responsible for LOH in human carcinogenesis (Fig. 2). However, the contribution of each chromosome alteration to the occurrence of LOH has been examined only for a few chromosomal regions in few tumor types. It was determined recently that 80% of LOHs are partial chromosome deletions

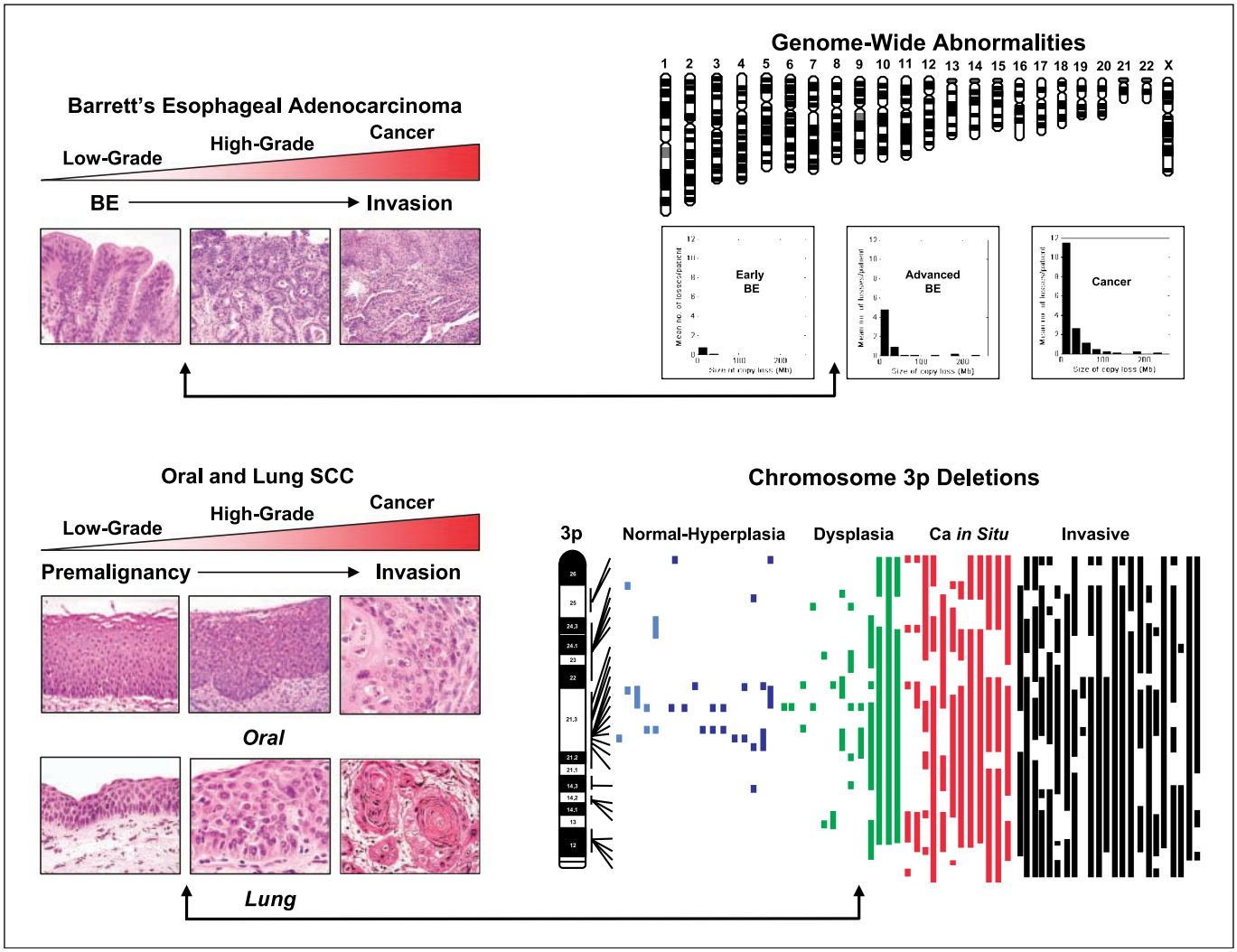

Fig. 1. Progression of premalignancy and molecular changes in Barrett's esophageal adenocarcinoma (*top*) and oral and lung squamous cell carcinomas (*SSC*; *bottom*). Chromosomal deletions at genome-wide and the 3p chromosome levels begin early in esophageal premalignancy (or BE) and, in oral premalignancy, progress and increase in size with increasing histopathologic severity (14, 15). Li et al. (15) showed that the number of larger copy losses (Mb) was significantly higher in advanced BE and esophageal adenocarcinoma compared with early BE lesions (*top*). In oral premalignancy progression, Tsui et al. (14) showed that the size of 3p segmental losses increased with histologic stage, as has been shown previously in progression to lung squamous cell carcinoma (*bottom*; refs. 24–26). In the lung squamous cell carcinoma model, discontinuous segmental losses, or LOH, at 3p are detected in normal epithelium, hyperplasia, and some dysplasias, whereas the whole arm is lost in invasive and *in situ* carcinomas and in a subset of dysplastic lesions; the vertical colored bars (*bottom right*) indicate the size of deletions; the gaps, or retentions, between the bars indicate discontinuous LOH. Histology pictures of BE progression are courtesy of Elizabeth Montgomery, M.D., and of oral leukoplakia progression are courtesy of Adel El-Naggar, M.D., Ph.D.

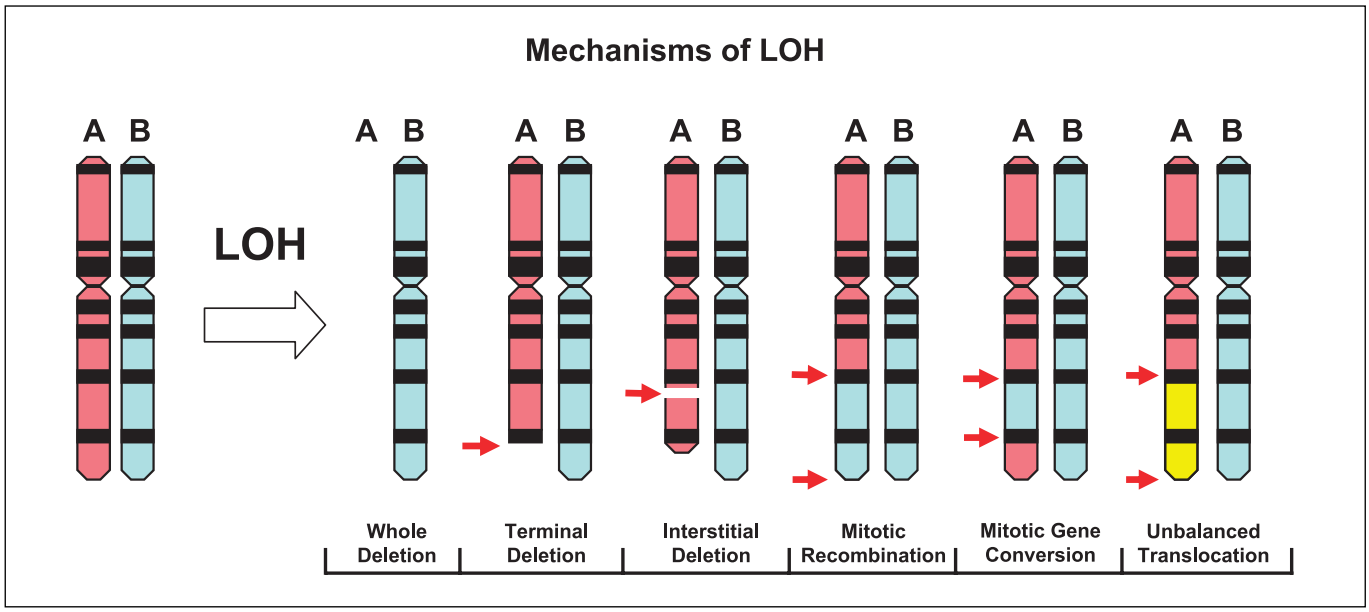

Fig. 2. Chromosomal alterations considered to be responsible for LOH in human carcinogenesis. The whole-chromosome, terminal, and interstitial deletions and unbalanced translocation represent copy number changes. The red arrows indicate sites of chromosomal breaks.

(involving the several chromosomal alterations mentioned above), whereas the remaining 20% were whole-chromosome deletions (33); these results came from integrating information on breakpoints for DNA copy number changes (obtained by a CGH) with numerical and structural chromosomal alterations (obtained by spectral karyotyping in lung cancer cell lines). We know that DNA copy number analyses of tumors have limitations due to the phenomenon that most of these alterations in invasive cancers are large, spanning many genes (tens to hundreds), including many that likely are not involved in oncogenesis. Therefore, studies of premalignant lesions with known outcomes are necessary to better define chromosomal loci and genes responsible for tumor development in humans.

The reports of Tsui et al. (14) and Li et al. (15) on the tumor models oral squamous cell carcinoma and Barrett's esophageal adenocarcinoma give new insights into and hope for understanding the highly complex nature of the progression of premalignancy to cancer. Despite their different pathogenetic features, these two tumors shared some common genetic progression characteristics. The application of high-throughput DNA technology in these models showed the promise of this approach and the merit of extending these methods to other premalignant diseases. It also showed the potential of CGH and SNP arrays to identify new candidate biomarkers and measures of clonal diversity, both of which can be used in patient management and assessment of cancer risk. Is less more? High-throughput approaches generate increasing amounts of data from smaller and smaller tissue samples, and chromosomal deletions appear to increase progression of premalignancy. These examples of "less is more" represent important advances in our understanding of the molecular nature of carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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