Editorial

A New Marker Determining Clonal Outgrowth

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Chronic exposure to common carcinogens such as tobacco and alcohol is the major cause of cancers in the upper aerodigestive tract including HNSCC, which includes cancers in the oral cavity, pharynx, and larynx with a worldwide incidence of >500,000 cases/year (1). Despite improvements in the diagnosis and treatment of HNSCC during the past few decades, patients with HNSCC continuously suffer high mortality and have poor quality of life. Recent research studies using molecular tools have identified many molecular abnormalities in HNSCC as well as in lesions in the early tumorigenic process (2). These advances extend our understanding of the biological nature of HNSCC and its development process at the molecular level.

Development of human cancer is a result of the accumulation of multiple genetic alterations leading to the evolution of clonal outgrowth and malignant transformation. Because the entire epithelial surface of the upper aerodigestive tract is exposed to common carcinogens such as tobacco and alcohol, it may be chronically damaged in a similar way and thus has an increased risk of developing multiple tumors over time (3, 4). Clinically, patients with HNSCC may present with multiple primary tumors or develop secondary primary tumors. Biologically, multiple tumors or premalignant lesions with distinct genetic and cytogenetic alterations have been reported in the oral cavity (5, 6). It is also true that multiple oral tumors or lesions in some individuals may be derived from a single progenitor cell (6, 7), although the mechanisms contributing to the phenomena remain unclear. Because of the distinct clinical outcome and treatment options for patients with recurrent tumors and those with second primary tumors, it is clinically important to determine the clonal origin of multiple head and neck lesions.

Several techniques have been used to determine clonal outgrowth in lesions with the potential to develop into invasive tumors. Microsatellite analysis measures LOH and microsatellite instability in clonal genetic alterations frequently found in HNSCC as well as head and neck premalignancies (8). LOH at critical chromosomal regions harboring tumor suppressor genes may be detectable in head and neck premalignancies, which indicates an increased risk of developing invasive tumors (9, 10). LOH and microsatellite instability have also been used as clonal markers to determine the origins of synchronous or metachronous lesions in the head and neck (6, 11). Because LOH frequencies are variable among chromosomal regions, and the informative (markers showing polymorphism) rates are also variable among markers, multiple markers must be used to gather the necessary information. Additionally, because there are three possible genetic patterns for any informative marker (i.e., retention, loss of upper allele, or loss of lower allele), about 33% of any pair of lesions may exhibit an identical genetic pattern by chance alone. To increase a high confidence in determination of clonal origin between two lesions, genetic patterns from multiple informative markers should be obtained. If two lesions share identical genetic patterns in a substantial number of markers, then they are likely derived from the same clone. However, a lack of shared genetic patterns in some markers between two lesions may occur even though the lesions derive from the same clonal origin because certain genetic changes may occur independently after the clone spreads out.

Specific mutations, such as those identified in tumor suppressor gene p53, have been used as specific genetic markers to distinguish the clonal relationship of multiple tumors in the upper aerodigestive tract (12). The probability of the same clonal origin is very high if tumors share an identical mutation in the gene. However, <50% of HNSCCs contain such mutations. The method is also limited because the mutations occur relatively late in the tumorigenic process. Therefore, it may not be suitable for analyzing multiple premalignant lesions, and even if different mutations are identified in two cancers, we cannot exclude the possibility that they have the same clonal origin.

Another approach to determine clonality and clonal origin is to analyze X chromosome inactivation status (13). Because one of two X chromosomes is randomly inactivated by DNA hypermethylation during early embryogenesis in females, a balanced X chromosome inactivation (DNA methylation) pattern between two X chromosomes is expected in normal tissues. When tissues with a clonal population are analyzed, hypermethylation should be detected only in one of the two X chromosomes. If two tumors/lesions show hypermethylation in different X chromosomes, then they must derive from different progenitors. If two tumors/lesions show an identical pattern of X chromosome hypermethylation, then there is a 50% chance that they have a common clonal origin. This approach has been used to determine the clonal origin of multiple HNSCCs (5). However, the methodology is limited to only female patients, who represent less than one-third of all patients with HNSCC in the United States (1), and certain technical difficulties such as incomplete enzyme cleavage...
due to variant CpG methylation status in the genome have been reported (14). Taken together, available markers are not sufficient to differentiate between second primary cancers and recurrent cancers in the head and neck for a substantial number of patients with multiple head and neck cancers/precancerous lesions.

In this issue of Clinical Cancer Research, Ha et al. (15) added a new marker to the arsenal for determination of clonal outgrowth. Ha et al. (15) analyzed head and neck premalignant lesions from 93 patients for deletions/insertions at a polycytosine tract (C-tract) in displacement loop (D-loop) of mitochondrial DNA and found such alterations in 37% of the lesions. Alterations at the C-tract were first reported in colorectal cancers by Habano et al. (16) and were considered a result of a defective DNA mismatch repair. The continuous increase of the deletion/insertion frequency of the C-tract from 22% in lesions with hyperplasia to 33% in lesions with mild dysplasia, 36% in lesions with moderate dysplasia, and 50% in lesions with severe dysplasia and 62% in carcinoma in situ (15) indicates that the alteration is associated with the severity of head and neck premalignancies. Ha et al. (15) proposed three possibilities for such an increase: (a) selective growth advantage; (b) defective cellular maintenance mechanism for DNA integrity; or (c) simply an increased chance due to clonal selection of a particular cell, as reported recently by Coller et al. (17). Regardless of what mechanism causes C-tract deletions/insertions in premalignant lesions, such deletions/insertions are unlikely to result in a significant biological consequence because the size of the C-tract is highly variable in healthy individuals (18). Nevertheless, the detection of such an alteration indicates a clonal outgrowth in the premalignant lesions. We, along with other research groups, have shown that a substantial number (but not all) head and neck premalignant lesions are clonal by LOH analysis and that LOH at critical tumor suppressor loci predicts malignant transformation (9, 10). The result from Ha et al. (15) is consistent with these previous observations and may reflect increases of true clonal lesions from hyperplasia to dysplasia. Because the C-tract alteration may not have a biological impact, its predictive value for malignant transformation remains to be determined.

Similar to the deletions/insertions measured by microsatellite analysis (6), the C-tract deletions/insertions appear to be good markers to determine clonal origin among multiple head and neck lesions (15). Because the rate of alteration in the C-tract is very high (up to 62% in carcinoma in situ), it may substantially reduce the complexity and cost of clonality analyses. It will be interesting to see whether combining the C-tract and other clonality analyses can substantially increase the informative rate and provide sufficient information to determine the clonal origin of multiple head and neck cancers/lesions in most patients. It should be noted, however, that the confidence of the same clonal origin between two lesions is lower when comparing lesions showing an identical 1-bp change at the C-tract to a specific mutation in a complex gene such as p53 because a 1-bp change in the C-tract accounts for two-thirds of all C-tract alterations (15, 19). David Sidransky’s research group (20) from Johns Hopkins has reported previously that specific homoplasmic point mutations may be frequently detected in the mitochondrial DNA of HNSCCs. Analyzing specific mutations in the mitochondrial genome might provide another tool in the determination of clonal origin among multiple cancers in the upper aerodigestive tract with a higher confidence. If these mutations occur early in tumorigenesis, they may also be valuable as markers to evaluate premalignant lesions.

References


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