Fluorescence Visualization in Oral Neoplasia: Shedding Light on an Old Problem

□□ *Commentary on Poh et al., p. 6716*

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In this issue of *Clinical Cancer Research*, Poh et al. (1) show the effectiveness of a simple handheld light (blue excitation light at 400-460 nm) to clarify the true extent of tumor spread and, in turn, guide complete removal of oral cancers in the operating room. Optical changes (specifically, loss of fluorescence) in the epithelium in and around oral cancers was used to map the "field of cancerization." Correlation with histopathologic features and specific genetic alterations indicate that fluorescence visualization is far superior to clinical judgment alone in gauging the size, extent, and distribution of the cancer field. Indeed, the field of optical changes seemed to more closely mirror the field of genetic changes than traditional light microscopy. This relatively simple approach of exposing subclinical alterations driven at the genomic level with lighting techniques and dyes such as toluidene blue could potentially find widespread practical application in operating rooms where definitive treatment of oral cancer relies on informed charting of the surgical boundaries.

More Refined Methods to Detect the Presence and Extent of the Oral Neoplasia Is the Key to More Effective Management of Patients with Oral Cancer

Carcinoma of the oral cavity is readily amendable to curative surgical removal; or so it first seemed to those pioneer surgeons who took on this disease in an earnest and focused way (2). The vast majority of oral cavity carcinomas, after all, take origin from a surface epithelium that is readily accessible to direct visual examination. At least in theory, oral cancer is a disease well suited for early detection and effective surgical intervention. Early optimism, however, has withered in face of the harsh reality that oral cancer is apparently not so easy to recognize and treat. The past 25 years has not seen a significant improvement in the early diagnosis of oral cancers, and most patients still do not present for diagnosis and treatment until they have stage III or stage IV disease (3). As a result, survival rates have remained unchanged for the 30,000 patients diagnosed in the United States each year with oral cancer.

Well-meaning appeals for routine oral examinations by qualified physicians and dentists may represent an overly simplistic reaction to a highly complex biological process. Time-honored methods that rely on clinical inspection and even microscopic examination to detect the presence and extent of the neoplastic process have been frustratingly ineffective when it comes to cancer of the oral cavity. Inexplicably, excised tumors recur in the face of microscopically free margins; patients effectively treated for oral cancer at one site develop second tumors at other sites; and premalignant lesions that regress during chemoprevention reappear and progress once therapy is halted. Clearly, novel methods are required that permit a more refined mapping of this elusive cancer spread beyond its clinical and even histologic boundaries.

Progression of Oral Cancer Is Driven by Genetic and Epigenetic Alterations

A description of the genetic changes driving tumorigenesis of the oral cavity provides a framework for better understanding perplexing patterns of tumor behavior. True to current models of tumorigenesis, the initiation and progression of oral cancer is driven by the accumulation of specific genetic and epigenetic alterations (e.g., promoter hypermethylation) that occur sequentially (4, 5). Some of the more common alterations target the p53 and p16 (INK4A)/retinoblastoma (Rb) pathways regulating cell growth. The p53 tumor suppressor gene (located on chromosomal arm 17p) is inactivated in >50% of oral squamous cell carcinomas; the p16 (CDKN2/MTS1) tumor suppressor gene (a key component of the Rb pathway, located on chromosomal arm 9p) is inactivated in up to 70% of oral cancers (5). The presence of these inactivating events can be inferred by the detection of loss of heterozygosity (LOH) targeting the chromosomal loci where they reside. Indeed, LOH involving chromosomal arms 17p, 9p, and 3p are among the most commonly observed genetic alterations observed in oral cancer.

LOH status at 17p, 9p, and 3p may serve as a useful biomarker for discerning patterns of tumor behavior. First, LOH at 3p and/or 9p represents an important step in driving a premalignant cell further down the path toward overt carcinomatous transformation. For oral leukoplakia, LOH at these loci is one of the more reliable predictors of clinical progression (6, 7). Second, LOH involving 3p, 9p, and 17p

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occur very early during oral tumorigenesis, sometimes even preceding the onset of discernable microscopic changes (8). LOH involving these loci have been used to track the presence and distribution of phenotypically normal but genetically altered cells throughout the oral mucosa.

A Genetic Description of Oral Tumorigenesis Has Redefined the Concept of "Field Cancerization"

Advances in the prevention, treatment, and surveillance of oral cancer await a more complete understanding of the mechanistic underpinning of tumor multifocality and local recurrence. According to the "field cancerization" concept proposed by Slaughter et al. (9) over four decades ago, multiple cell groups independently undergo neoplastic transformation under the stress of regional carcinogenic activity. Recent genetic approaches to the study of oral cancer have challenged the notion that independent transforming events are commonplace for individual patients. Indeed, when one oral cancer is compared with a second synchronous or metachronous oral cancer, the paired tumors often harbor identical patterns of genetic alterations (i.e., concordant patterns of LOH at 3p, 9p, and 17p), indicating origin from a the same genetically altered field (10–12). Presumably, a critical genetic alteration in a progenitor cell provides a growth advantage over its neighboring cells. All subsequent daughter cells share this initiating genetic event. At some point after transformation, cells harboring these early genetic alterations migrate or simply overpopulate contiguous tracts of mucosa. As the process further evolves, the accumulation of other independent genetic alterations confers an additional growth advantage to subpopulations of cells. Ultimately, dominant outgrowths of more aggressive phenotypes give rise to multiple tumors at discontinuous sites within the oral cavity.

Visualization of "Field Cancerization" Is Not Reliable by Routine Direct Visual Examination or Even Microscopic Examination

Not only is the mechanism by which genetically altered cells migrate to populate extended tracts of the oral epithelium poorly understood, but also their spread is not easily appreciated by routine visual examination or even microscopic examination. In the upper and lower respiratory tracts, the notion that the accumulation of genetic damage is invariably accompanied by predictable morphologic patterns has been repeatedly challenged by observations of widespread genetic alterations in the absence of histopathologic changes of dysplasia or malignancy (13). This phenomenon has profound implications on time-honored management practices. As one example, the traditional definition of a "negative" surgical margin now demands redefinition in light of the propensity of genetically damaged cells to populate extended tracts of histologically normal oral mucosa. Indeed, the presence of a histologically normal but genetically altered cells at the surgical margin as detected by p53 mutational analysis or microsatellite analysis is a strong predictor of local tumor recurrence (14–17).

In the intraoperative setting, the potential usefulness of genetic assessment as a means of guiding the extent of surgical resection is offset by practical concerns relating to the timeliness of results. In the past, genetic analysis of surgical margins required days to weeks using traditional detection assays. More recently, replacement of these cumbersome and time-consuming assays by highly streamlined semiquantitative methods for detecting low-level genetic and epigenetic alterations has dramatically reduced turn-around times. For resection of head and neck cancers, genetic margin assessment can now be completed in just <5 hours (18, 19), a vast improvement over traditional methods but still beyond the expectations and patience of most head and neck surgeons. There remains an urgent need for simple, inexpensive, and objective methods that can provide real-time results in the intraoperative setting for the detection of oral neoplasia.

Assisted Visualization as a Means to Establish the Presence and Extent of Oral Neoplasia

Assisted visualization of oral neoplasia in the intraoperative setting is being developed along several lines. Some have advocated staining the oral mucosa with the vital dye toluidine blue as a means of visualizing the presence of subclinical disease (20, 21). Abnormal staining of the oral mucosa has been shown to correlate with the presence of histologic dysplasia, the presence of LOH, and increased cancer risk (20–22). Others have explored visual assessment of oral cavity luminescence following a chemical reaction (i.e., chemiluminescence) as a means to discriminate between normal and neoplastic mucosa (23). Still others have used blue excitation light (400-460 nm) to discern the presence of subclinical oral neoplasia (24, 25). Although the precise mechanisms are not well understood, changes in the metabolic activity of the surface epithelial cells induce spectral changes that impart "tissue fluorescence signatures." At certain wavelengths, premalignant lesions of the oral cavity show less fluorescence than surrounding normal oral mucosa. Given this enhanced ability to directly visualize premalignant lesions, fluorescent visualization has been use in some populations at risk for developing squamous cell carcinoma of the lung (26, 27), oral cavity (24, 28), and other sites (29).

Poh et al. (1) use fluorescence visualization, not as a screening tool but as a means of delineating the surgical boundaries in the intraoperative setting. One might argue that this method does not advance the intraoperative frozen section approach—a time-honored technique for identifying premalignant changes at the surgical margins—but a few differences are worth noting. First, the frozen section is a sequential process that provides non-instantaneous results, whereas autofluorescence visualization provides real-time results. Second, the frozen section requires communication between the pathologist and surgeon, whereas autofluorescence visualization facilitates direct assessment by the surgeon without the need for a liaison. Third, the frozen section provides linear analysis of a segment of the surgical boundary, whereas autofluorescence visualization provides a topographical view of the entire neoplastic field including its spatial relationship to various anatomic landmarks. Finally, autofluorescence visualization may more fully encompass the field of genetically altered cells (Fig. 1). LOH at 3p and/or 9p, genetic alterations that predict local tumor recurrence, were detected at the periphery of the optically altered fields often in the absence of any corresponding histologic changes.

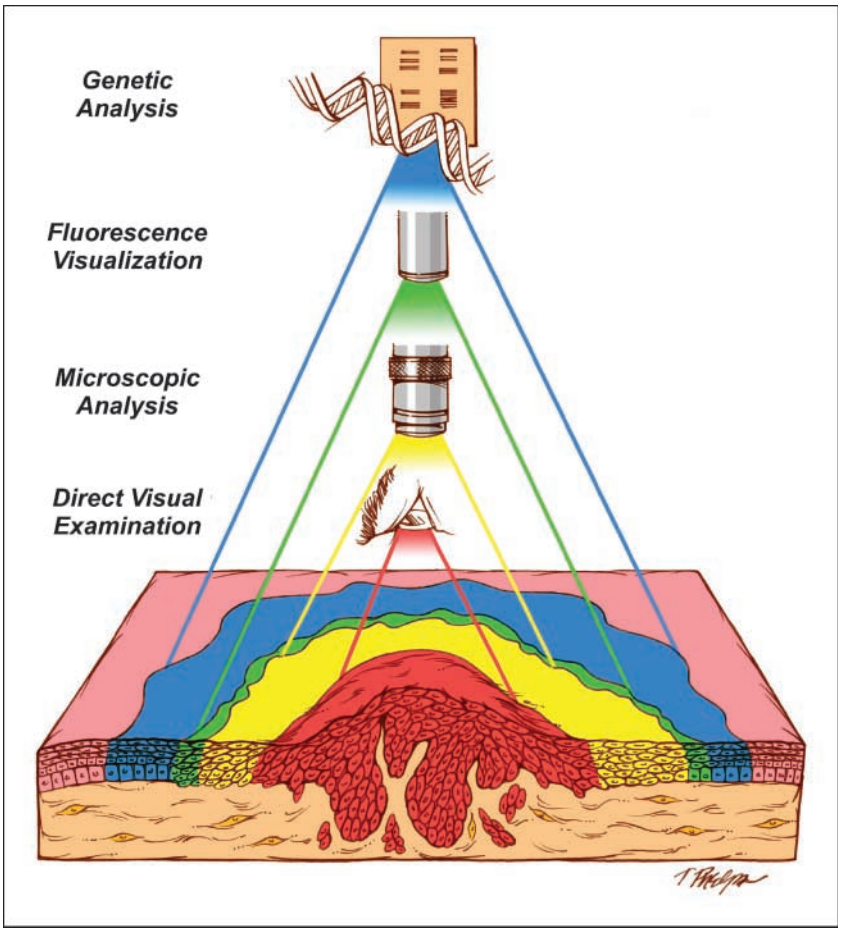

Fig. 1. Invasive oral cancers arise from genetically altered cells distributed throughout tracts (i.e., "fields") of oral mucosa. The aim of surgery is to remove the entire tract neoplastic field, but the ability to discern its boundaries in the operating room depends on the method of detection. Direct visualization examination (*red zone*) underestimates the full extent of histologic alterations as detected by intraoperative frozen section histology (*yellow zone*). Fluorescence visualization (*green zone*) is a simple and real-time method for visualizing optical changes associated with subclinical premalignant disease. The boundaries established by fluorescence visualization may even extend beyond the zone of histologic changes to more fully encompass the field of genetic alterations (*blue zone*). (Reprinted with permission from John Hopkins Medical Institutions © 2006).

As advocated by Poh et al. (1), autofluorescence visualization is a method that is no more complicated than operating a simple handheld device. Whether this approach can shed new light on highly complex biological processes and perplexing patterns of clinical behavior awaits (*a*) analysis of larger sample sizes; (*b*) more extensive histologic and genetic mapping of optically altered mucosa; (*c*) a more comprehensive analysis of factors likely to affect the optical qualities of the oral mucosa such as inflammation and prior treatment (e.g., chemotherapy, radiation therapy); (*d*) careful correlation with clinical variables; and (*e*) a head-to-head comparison with other promising detection methods such as visualization of oral mucosa luminescence at wavelengths that do not require a special apparatus (23) and the highly specific toluidene blue staining method (22). These extended efforts to rigorously test the acuity of various detection methods are worthwhile as definitive surgical removal of oral cancer depends on the ability to recognize oral neoplasia during its early stages and to discern its distribution throughout the oral cavity mucosa.

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