

Editorial

Toward Early Oral Cancer Detection using Gene Expression Profiling of Saliva: A Thoroughfare or Dead End?

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As early as the beginning of the 20th century, squamous cell carcinoma (SCC) of the oral cavity was perceived to be a disease on the verge of eradication, a grand optimism buttressed on the presumptions that the oral cavity was readily accessible to inspection, that neoplasia of the oral cavity could be easily recognized during early stages of tumor progression, and that early detection would permit complete and curative resection (1). A century later, oral SCC persists as a formidable clinical challenge. The notion that early stages of oral tumorigenesis can be easily visualized and halted now appears too simplistic. Startling new observations suggest that the clinical and histologic appearance of the oral mucosa may not fully disclose the scope of damage at the genetic level (2). This phenotypic and genotypic disparity may account, in part, for the failure to establish effective screening and surveillance protocols based on traditional clinical and microscopic examination.

True to current models of tumorigenesis, the initiation and progression of oral SCC is driven by the accumulation of specific genetic alterations. Understanding the molecular underpinning of oral SCC is providing a more complete picture of the ways in which these tumors arise and advance and is providing a rationale for novel strategies of cancer detection. To date, those strategies have focused primarily on tumor-specific DNA alterations in serum and body fluids as a means of early cancer detection. The oral cavity has been identified as a site particularly conducive to such strategies, given the ease with which fluids and exfoliated cells can be collected.

The work of Li *et al.* (3) published in this issue of *Clinical Cancer Research* represents another step forward in this promising endeavor. It consists of a phase II detection trial to distinguish patients with oral cancer from controls. Li *et al.* (3) show that tumor-specific genetic profiling is not to be restricted to patterns of DNA damage. RNA can also be salvaged from saliva (a medium generally regarded as entirely inhospitable to RNA), forging a path for saliva-based gene expression profiling.

For biomarkers to be truly useful in early cancer detection, they must meet established criteria (4): (a) they must be altered in such a way as can be objectively measured; (b) they

must be measurable even in small specimens; (c) they must be altered in high-risk tissues, but not in normal tissues; and (d) they must be altered in the early stages of cancer development. The work of Li *et al.* (3) removes any doubt regarding the first two of these criteria: RNA-based analysis of body fluids is indeed possible, even with fluids presumed to be hostile to RNA integrity. The full power of gene expression analysis can now be brought to bear on readily accessible saliva samples. The latter two criteria are more difficult to realize. Their elusiveness does not so much expose the weakness of this and other studies as underscore the inherent difficulty in establishing the specificity and timing of potential biomarkers. Overcoming these obstacles requires some difficult yet necessary steps.

First, selection of the most discriminatory and predictive biomarkers requires robust validation in large and unbiased groups of patients. The expression profile endorsed by Li *et al.* (3) was inferred from a small number of patients, and the cohort used to establish the cancer-specific profile was inappropriately included in the cohort used to validate the profile.

Second, the exclusivity of a gene expression profile for oral neoplasia must first be established before that particular profile is embraced as a “cancer signature.” The background frequency of the biomarker must be documented for individuals without oral cancer across a broad range of exposures (*e.g.*, tobacco and alcohol) and nonneoplastic conditions (*e.g.*, dental caries and gingivitis). The enthusiasm for using interleukin (IL)-8, an inflammatory cytokine, as a specific biomarker of oral cancer must be tempered by an awareness that IL-8 levels also increase in a variety of oral inflammatory conditions (5, 6). IL-8 measurement has been advocated as an effective means of monitoring oral disease activity ranging from dental caries to chronic aphthous ulcers to (now) oral carcinoma. Ongoing population-based screening studies that seek to resolve cancer-specific alterations from the background clamor of extraneous variations will ultimately prove indispensable to a more deliberate interpretation of gene expression data. This uncompromising rejection of even a marginal false positive rate has been the downfall of many initially promising attempts to screen for oral cancer.

Third, translational studies that focus on early cancer detection and cancer risk assessment cannot lose sight of oral premalignancies (*i.e.*, oral dysplasia and carcinoma *in situ*), not overt malignancies, as the optimal target of gene expression profiling. Because Li *et al.* (3) did not include premalignancies, effective application of their approach to the arena of oral cancer screening is intriguing but speculative. Cancer-specific expression profiles of clinically apparent carcinomas are diagnostically relevant only to the degree that they are consistently present and measurable in early (*i.e.*, preclinical) stages of tumor progression. The initiation of phase III detection trials that involve the

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collection of individuals with clinically silent oral neoplasia is imperative to translational research in oral cancer detection but exceedingly difficult. The group of individuals with oral dysplasia/carcinoma *in situ* is very small; these individuals do not tend to seek out health care professionals; and, if they did, they would likely encounter health care workers poorly trained and ill-equipped in the recognition of premalignant changes.

The work of Li *et al.* (3) opens a new avenue for the early detection and intervention of oral cancer. However, the road to practical and effective oral cancer screening based on gene expression profiling is a long one, and Li *et al.* (3) bring us only a few small steps further along it. Progress along this road will be ultimately propelled by the identification of biomarkers with a definite specificity for oral neoplasia (inclusive of its early stages) as validated in large populations of individuals without oral cancer, with oral cancer, and at high risk of developing oral cancer.

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