Published OnlineFirst August 26, 2010; DOI: 10.1158/1940-6207.CAPR-10-0173

Perspective
Perspective on Pattani et al., p. 1093

Cancer Prevention Research

Screening for Oral Premalignancy and Cancer: What Platform and Which Biomarkers?

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Abstract

This perspective on the report by Pattani et al. in this issue of the journal (beginning on page 1093) examines the utility of detecting hypermethylation of the candidate tumor suppressor genes endothelin receptor type B (*EDNRB*) and kinesin family member 1A (*KIF1A*) as a means of oral cancer or premalignancy screening. The data discussed here raise the possibility that saliva-based hypermethylation studies may hold promise as a cancer screening platform. This perspective also discusses some of the challenges and current limitations of developing biomarkers to screen not only for oral premalignancy and early cancer but for human papillomavirus–related oropharyngeal neoplasia as well. *Cancer Prev Res; 3(9); 1056–9. ©2010 AACR.*

With an annual incidence of nearly 600,000 cases, head and neck cancer, which comprises oral squamous cell carcinoma (OSCC) and oropharyngeal and pharyngeal SCC, is the sixth most common malignancy in the world today (1). Chronic exposure to tobacco products and alcohol is the classic and most common etiologic factor for OSCC (2). Although present in only less than 5% of OSCC, high-risk forms of the human papillomavirus (HPV) are another causative factor and are present in a growing proportion of patients with oropharyngeal malignancies (3–8). Despite numerous advances in therapy, the long-term survival of patients with OSCC, particularly OSCC associated with classic etiology, has remained modest. Several factors contribute to this poor outcome. First, OSCC is often diagnosed at an advanced stage. The 5-year survival rate of early-stage disease is ~80% and that of late-stage disease is only ~20% (9). Second, "field cancerization" leads to the development of multiple primary tumors with a major impact on survival. Second primary tumors are the most common cause of treatment failure and death in patients with early-stage disease (10, 11). Therefore, comprehensive treatment plans must include improved forms of both screening and chemopreventive strategies to improve long-term outcomes.

Cancer screening can be defined as testing asymptomatic individuals to sort out those who probably have the disease from those who probably do not. Depending on the neoplasia in question, screening (e.g., in the cervix, colon, and oral cavity) may involve the detection of both invasive and precursor (premalignant) lesions. It is generally accepted that screening for OSCC and oral premalignant lesions may decrease the devastating morbidity and mortality associated with this disease. In contrast to skin cancer screening, however, where visual examination has sensitivity and specificity rates of 93% and 98%, respectively (12, 13), the detection of OSCC and premalignant oral lesions by way of the conventional visual and tactile examination (CVTE) has remained problematic for several reasons. First, oral premalignant lesions and some early OSCC can be subtle in clinical appearance and can mimic inflammatory lesions. Second, it is becoming increasingly clear that some precancerous lesions are not readily identified during a conventional visual examination using incandescent light (14). Therefore, adjunctive screening aids (devices or tests) that can improve the diagnostic accuracy of the CVTE are desperately needed.

From the perspective of everyday clinical practice, the most beneficial screening adjuncts would be those that help the clinical decision-making process in managing visible oral mucosal lesions and/or help to detect clinically invisible premalignant disease that lurks within the oral mucosa. With advancing technologies, the field of screening adjuncts for oral premalignancy and OSCC has blossomed over the last decade. In a broad-strokes view, the following platforms currently receive the greatest attention and have the greatest clinical potential: light-based handheld devices; cytology with or without additional analyses (e.g., loss of heterozygosity and spectral imaging); *in vivo* imaging with molecular probes/paints; and salivary diagnostics.

Commercially available handheld wide-field devices emit variable wavelengths of light that can result in reflectance and/or autofluorescence of the oral mucosa (15–17). These screening adjuncts were cleared for marketing under the Food and Drug Administration 510K paradigm, which does not require the demonstration of efficacy. They have been heavily marketed to the medical

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doi: 10.1158/1940-6207.CAPR-10-0173

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and dental communities as an inexpensive and rapid way of improving the CVTE. Owing to their clearance under the 510K paradigm, however, there are limited published data supporting their ability to increase diagnostic accuracy or assist the decision-making process for clinically evident lesions. Furthermore, although reported anecdotal observations and small case series support the utility of these handheld devices, no appropriately designed and sufficiently powered studies to establish efficacy in identifying premalignant lesions not readily identified by the naked eye have been reported. There is also considerable interest in spectroscopy (narrow field) for identifying oral premalignancy and OSCC (18–21). This technology uses a small-diameter probe placed in direct contact with the oral mucosa to obtain a spectral reading. With its limited field, spectroscopy may be impractical in general clinical screening practice. It may be very useful, however, in the setting of discrete lesions of clinical concern that are detected by CVTE.

Most other OSCC and oral premalignancy screening adjuncts are based on biomarker assessments (22–25). Regardless of the platform, the efficacy of these adjuncts ultimately will depend on establishing biomarkers with a sufficient degree of specificity and sensitivity to indicate the prognosis of a given lesion(s). This requirement is particularly true of screening for oral premalignancy. It has long been appreciated that the majority of histologically premalignant oral lesions do not progress to cancer. Clinical experience also has taught us that some histologically benign lesions are actually molecularly premalignant and progress to cancer. The obvious question is "Which are which?" The inability of conventional histopathology to stratify lesions with a sufficient degree of specificity, sensitivity, and positive predictive value further underscores the need for molecular-based biomarkers.

As reported in this issue of the journal (26), Pattani et al. investigated promoter hypermethylation of the candidate tumor suppressor genes endothelin receptor type B (*EDNRB*) and of kinesin family member 1A (*KIF1A*) in salivary rinses [normal saline solutions that had been gargled and used to rinse the oral cavity and to which were added exfoliated cells collected by swabbing (with cotton-tipped applicators) the buccal mucosa, alveolar ridge, lateral tongue, floor of mouth, and pharyngeal inlet]. They then asked whether the association of the methylation status of these genes, combined with histologic diagnosis and risk classification, improved diagnostic accuracy. Using quantitative methylation-specific PCR, the authors found that hypermethylation of *EDNRB* combined with clinical risk assessment slightly improved sensitivity for identifying early-stage OSCC or dysplastic oral premalignant lesions, from 71% (clinical alone) to 75% (combined). These data suggest that quantitative methylation-specific PCR is a rapid and reliable method for determining the methylation status of genes in oral mucosal cells derived from saliva. Furthermore, they suggest that combining clinical risk assessment with identifying hypermethylated genes may be a useful platform for oral premalignancy and/or OSCC screening.

Certain limitations of this study, however, underscore many of the current challenges associated with the development of validated biomarkers for cancer screening. First, although adding *EDNRB* methylation status slightly increased the sensitivity of clinical risk assessment, the combination also resulted in decreased specificity and positive predictive value. Furthermore, the receiver operating characteristic curves showed that the combination resulted in a modest improvement of the area under the curve from 0.65 to 0.68, reflecting little to no increase in predictive power. To have a clinical benefit, a diagnostic test must provide clear scientific evidence that it assists the treatment decision-making process; a seminal article by Jaeschke et al. (27) provides an appropriate discussion and clinical decision-making framework for clinicians when considering whether or not to perform a particular test. Therefore, although the use of saliva-based queries of tumor suppressor gene hypermethylation continues to hold great promise for oral premalignancy and/or OSCC screening, the modest improvements in prediction afforded by using *EDNRB* as a stand-alone biomarker make the diagnostic utility of this test unclear at this juncture. Alternatively, it may be possible to increase the diagnostic utility of *EDNRB* hypermethylation by combining it with additional candidate genes.

Furthermore, Pattani et al. were hampered in this study by a critical issue that challenges all investigators working in the field of oral premalignancy and/or OSCC screening and biomarker development. Namely, they did not have a large enough collection of biospecimens associated with long-term patient follow-up to provide sufficient statistical power to validate that the biomarkers they evaluated meet the gold standard for this class of biomarkers (i.e., the ability to define the risk of progression to malignancy). This standard is the keystone of a biomarker's clinical utility. Well-annotated biospecimens are the norm today in basic and translational research of cancer but not of premalignancy. The collection of biospecimens with long-term follow-up information from patients with premalignant disease is extremely difficult to obtain for several reasons including their low rate of malignant transformation (thus necessitating larger cohorts of patients), the heterogeneous manner in which premalignant lesions are currently managed, and the potentially long duration between the initial diagnosis and the development of OSCC (which can be as long as 10 years).

Therefore, several steps must be taken to help address this issue. First, every effort should be made to develop multi-institutional oral premalignancy and/or OSCC screening clinical trials that carefully address the issues of biomarker validity; comparison to the current, though imperfect, gold standard of histopathologic analysis; appropriateness of the patient population; the use of proper study clinicians; specificity; and potential for replication. Second, we should establish quality assurance and quality control mechanisms that ensure accurate data

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collection. Last, a concerted effort is needed to collect, store, and equitably distribute high-quality, well-annotated human biospecimens. This framework would greatly facilitate oral premalignancy and/or OSCC screening research and would create a highly collegial environment that would be ready for collaborative work that will establish oral premalignancy biomarkers meeting the gold standard of predicting cancer.

Finally, a growing concern of this field is the increasing incidence of HPV-related oropharyngeal SCC. To address this alarming increase, we need better prevention and screening for oropharyngeal cancer and premalignancy, which raises a host of issues. Whereas oral premalignant lesions (e.g., leukoplakia) of classic etiology (tobacco, alcohol) are well described, the clinical appearance of HPV-related premalignant oropharyngeal lesions has not been adequately described. Furthermore, oropharyngeal SCC is often difficult to observe during a clinical examination because it may originate in hard-to-examine locations (e.g., a tonsillar crypt in cases of HPV-related lesions). Developed for classic oral lesions, handheld devices and other adjuncts have no published data with regard to their utility in screening for HPV-related oropharyngeal lesions. Similarly, diagnostic biomarkers under investigation for head and neck cancer screening have been largely derived from patients with classic OSCC. Given recent work showing that the gene expression profiles of classic OSCC and of HPV-associated oropharyngeal SCC are considerably different, it is likely that screening in the two sites will require two different sets of biomarkers (28, 29). The biomarker set for oropharyngeal screening will need to cover HPV-related and tobacco/alcohol-related neoplasias, both of which can occur in smokers. One future avenue of study may be to examine methylation markers in HPV-related oropharyngeal lesions. Biomarker assays for neoplasia in the oral cavity or oropharynx may be diluted in salivary rinses, which collect cells from both sites; salivary rinses also may not collect enough oropharyngeal cells to meet cutoffs for positive biomarker findings in any case. Scrapings, which are site specific, would help overcome these obstacles. Platforms and biomarkers for neoplasia screening in the oral cavity and oropharynx must be carefully validated before their efficacy can be defined.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received 07/26/2010; accepted 07/26/2010; published OnlineFirst 08/24/2010.

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Cancer Prev Res 2010;3:1056-1059. Published OnlineFirst August 26, 2010.