PERSPECTIVE

A Novel Target for Oral Cancer Chemoprevention? Notch Quite, Yet…

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Abstract
The two major goals of oral cancer chemoprevention efforts are the ability to segregate the high-risk patients and the identification of an effective pharmacologic agent that halts progression to invasive cancer. Considerable progress has recently been achieved in profiling invasive head and neck squamous cell carcinomas, particularly with the use of high throughput technologies. Similar molecular characterization of potentially malignant oral epithelial lesions (OPMLs) [leukoplakia and erythroplakia] is yet to be accomplished. It is postulated, though, that molecular profiling could lead to the discovery of novel markers of cancer risk that could also serve as potential targets for chemoprevention. In this perspective, we comment on the work by Izumchenko et al. that reports a high prevalence of NOTCH1 gain of function mutations in Chinese patients with OPMLs. Although additional studies are needed to validate the findings, the study is the first to link alterations in this gene in oral premalignancy. These findings could serve as a first prototype of a single gene mutation as a potential target in clinical chemoprevention setting.

Key words: oral premalignant lesions, leukoplakia, chemoprevention, NOTCH1
Introduction

Central to successful oral cancer chemoprevention efforts are identification of a high-risk subset and finding a pharmacologic agent that can effectively halt the process of oral carcinogenesis. Oral potentially malignant lesions (OPMLs) are clinically recognized as leukoplakia and erythroplakia. However, accurate prediction of their progression within and between subjects is currently elusive. A variable risk of malignant transformation of leukoplakias has been reported in the literature, with geography (and most likely etiologic factors) playing a major role in influencing the incidence of invasive cancer development. In a populational-based study in India (where betel nut chewing and use of smokeless tobacco is very prevalent), the rate of malignant transformation was reported at 0.06% per year (1), whereas this figure seems to be higher in hospital-based studies in the Western world (2). The presence of dysplasia of moderate to severe degrees is commonly used in risk assessment and as an indication for surgical resection of leukoplakias. This strategy, however, is suboptimal at several levels: the correlation between dysplasia and subsequent invasive cancer development is not perfect – there is great variability in the classification of dysplastic lesions among pathologists, and surgical resection may not address the remainder of the mucosa exposed to the field-cancerization effect still at risk for oral cancer (3).

The underlying molecular events of oral carcinogenesis have been the subject of intense investigation. In 1996, Califano et al. proposed a genetic progression model of oral cancers by analyzing ten tumor suppressor loci in oral lesions histologically characterized as hyperplasia, dysplasia, carcinoma in situ and invasive oral cancers.
According to this model, activation of oncogenes and inactivation of tumor suppressor genes lead to progression of premalignant lesions to invasive cancers; specific genetic events may occur in a distinct order of progression; and the accumulation of these genetic events, rather than the order in which they occur, ultimately determines malignant transformation (4).

Similarly, a transcriptional progression model has been developed, demonstrating a clear separation in gene expression profiles between histologically normal mucosa, premalignant lesions, and invasive head and neck squamous cell carcinomas, with a close association of the transcriptome alterations between the latter two groups (5). However, a limitation of these previous studies was the small size and the inadequate sequential temporal sampling of progressive lesions. Although a temporal relation can be inferred on the basis of the histological appearance of the lesions, little is known about the associations of these OPML molecular alterations with cancer risk and their active participation in the process of malignant transformation (as opposed to being just passenger events and/or a reflection of chromosomal instability). Nonetheless, these data provide a framework for further studies on the molecular evolution of OPMLs as they progress to invasive cancers, leading to potential discoveries on novel targets for oral cancer chemoprevention.

To improve risk stratification and clinical management of oral premalignancy, several groups have investigated the role of molecular markers in determining their probability of progression into invasive cancer. A potential marker of cancer risk identified in these lesions is loss of heterozygosity (LOH) at certain chromosomal sites harboring tumor suppressor genes (e.g., 3p14, 9p21). Based on the high frequency of
LOH in invasive cancers, Mao et al. reported the incidence and the prognostic significance of LOH at 3p14 and 9p21 in oral leukoplakia. The authors demonstrated that LOH at these loci was associated with a 5-fold increase in the risk of invasive cancer development (6). Subsequent studies have confirmed these findings (7–9).

Based on these seminal data, we launched and recently completed the first randomized, oral cancer chemoprevention clinical trial that included molecular risk stratification as an integral eligibility criterion – the Erlotinib Prevention of Oral Cancer (EPOC) study. In EPOC, patients with OPMLs (with or without a prior history of oral cancer) were tested for LOH at 3p14, 9p21 and additional chromosomal sites. If high-risk LOH profiles were identified, patients were randomized to receive placebo versus erlotinib for 12 months, with a primary endpoint of oral cancer-free survival. LOH-negative patients were not treated on study but monitored for oral cancer development. EPOC provided, for the first time, prospective validation of LOH as a marker of oral cancer risk, within the context of an interventional clinical trial – at 3 years, oral cancer-free survival was 76% of patients with LOH-positive lesions versus 87% for the LOH-negative group (p=0.01). Erlotinib did not improve oral cancer-free survival in the study population overall (10). One of the main limitations of EPOC was the dissociation between the marker of cancer risk utilized as the eligibility criterion (LOH) and the molecular target (EGFR) for the intervention (erlotinib). Of note, EGFR protein overexpression and gene copy number gain in OPMLs have been described as potential markers of malignant transformation (11), and ongoing, subgroup analysis of EPOC will determine if erlotinib is effective in reducing the incidence of oral cancer in the EGFR-positive subgroups. These shortcomings of the EPOC trial illustrate the need
to identify novel prognostic markers in OPMLs that [1] are fully characterized preclinically and clinically, in regards to their role in oral carcinogenesis, and [2] can not only help predict the risk of invasive cancer, but also serve, at the same time, as targets for pharmacologic intervention.

The availability of high throughput genomic analysis and their applications in head and neck squamous cell carcinomas (profiling for the most part oral cavity cancers) has provided detailed information on the genomic spectrum of these tumors. The most common somatic mutations identified in one of the initial high throughput sequencing studies of invasive head and neck cancers occurred in the genes TP53, NOTCH1, CDKN2A, PIK3CA, FBXW7, and HRAS at frequencies of 47, 15, 9, 6, 5, and 4%, respectively. Of the 28 NOTCH1 mutations observed, 11 were predicted to truncate the protein product, and therefore probably represent inactivating mutations, whereas 17 were missense mutations (12). These findings were, for the most part, recapitulated in independent cohorts (13–15). As genomic characterization efforts evolve, geographic regional differences in the frequencies of mutations have been reported, which may reflect the influence of variable etiologic and environmental factors on the development of head and neck squamous cell carcinomas. As an example, the India Project Team of the International Cancer Genome Consortium identified five new genes frequently altered (at a rate of 10–22%) in gingivo-buccal squamous cell carcinomas not previously reported in Western studies – USP9X, MLL4, ARID2, UNC13C and TRPM3. Use of smokeless tobacco was nearly universally present in this cohort, which could explain some of the differences compared to previous reports (15). Likewise, Song et al. recently reported a frequency of 43% of nonsynonymous NOTCH1 somatic mutations in
51 Chinese patients. In 36% of the patients with mutated tumors, the \textit{NOTCH1} mutations were localized to the heterodimerization domain, where most of the activating mutations in hematologic malignancies occur, in sharp contrast to the \textit{NOTCH1} (inactivating) mutation findings in sequencing studies in Caucasian patients. It was postulated that use of high alcohol-containing beverages in China could account for some of these differences (16).

In this issue of \textit{Cancer Prevention Research}, Izumchenko et al. reports on the prevalence of \textit{NOTCH1} mutations in Chinese patients with oral leukoplakia and invasive oral cancers. They demonstrated that 54% of the primary squamous cell carcinomas, and 60% of the OPMLs carried a \textit{NOTCH1} mutation. Consistent with the findings previously described by Song et al., a substantial proportion of the \textit{NOTCH1} mutations identified in this defined ethnic cohort were postulated to confer gain of function. The authors concluded that \textit{NOTCH1} mutations are therefore bona fide drivers of oral carcinogenesis (17). While an exciting finding, the potential clinical relevance must await further independent validations in different geographic populations.

For future validation of these findings, samples collected at different time points along the course of progression of OPMLs in the same patients should be assayed. Arguably, LOH at 3p14 and 9p21 have been embedded into prospective clinical trials because of their robust association with oral cancer risk, and it is expected that confirmation of similar data would be optimal in order to move forward with \textit{NOTCH1}-based strategies to either stratify and/or treat OPML patients within the context of chemoprevention studies; the mere presence of \textit{NOTCH1} mutations at similar frequencies in OPMLs and invasive cancers is insufficient to classify this molecular alteration as a driver event. In
the absence of mechanistic preclinical studies specific to oral carcinogenesis and information on the invasive oral cancer risk conferred by NOTCH1 mutations in OPMLs, there is only limited data to support their role as a driver versus passenger event. On the other hand, the fact that Chinese patients with invasive oral cancers with NOTCH1 mutations have a worse prognosis (16) is a strong argument for additional studies to further characterize these molecular abnormalities from a clinical and laboratory standpoint in the setting of oral pre-malignancies.

Despite these limitations, the work by Izumchenko et al. carries significant implications to the head and neck cancer chemoprevention field. NOTCH1-activating mutations are present in 50% of patients with T-cell acute lymphoblastic leukemia (T-ALL; ref. 18). Aggressive forms of chronic lymphocytic leukemia and mantle cell lymphoma also exhibit NOTCH1 mutations (19, 20). Two main strategies have been developed to target NOTCH1 in the clinical setting: gamma secretase inhibitors and anti-NOTCH1 antibodies. Preclinical data have demonstrated activity of NOTCH1 inhibitors in hematologic malignancies (20–22), and these drugs are now being evaluated in clinical trials, with early signs of activity in T-ALL (23). Challenges of developing these drugs in the clinical setting have been described elsewhere (24) and include determination of optimal dosing schedule and management of gastro-intestinal toxicities. However, if these drugs are confirmed to have positive clinical effects in hematologic malignancies with NOTCH1-activating mutations, this could open up a pathway for drug repurposing for management/prevention of oral cancers. Accordingly, if the NOTCH1-mutation findings described in Chinese patients with OPMLs are confirmed to represent a driver event and to confer high risk for oral cancer, this could
represent the first report of a targetable gain-of-function mutation that actively participates in the process of oral carcinogenesis. One could easily envision a chemoprevention strategy according to which patients with OPMLs would be sequenced for the NOTCH1 gene, and if an activating mutation were identified, they would be exposed to a NOTCH1 inhibitor. While, at this time, it is unclear whether this would only be applicable to ethnic Chinese populations, this could conceivably be the first example, in oral cancer prevention, that a possible marker of cancer risk and driver of carcinogenesis is targeted to suppress invasive cancer development. This clearly addresses the aforementioned EPOC trial limitation, characterized by the risk marker-target dissociation.

The work by Izumchenko et al. also illustrates the need for extensive molecular profiling of OPMLs in an attempt to improve chemoprevention strategies. We envision that the following steps will be key to streamline drug development in this field:

1. Sequencing of large cohorts of clinically annotated specimens, with long term follow up and information on ethnicity, environmental and tobacco / alcohol exposure to identify other potential markers of cancer risk that can be targeted in a personalized way;

2. Analysis of the temporal progression of molecular changes within and across patients;

3. Integrative multi-platform studies of the molecular pathways in OPMLs to identify pharmacologic strategies targeted at key driver events.
In summary, next generation sequencing and high-throughput molecular profiling of OPMLs with novel technologies have the potential to open up opportunities for novel chemoprevention strategies, as illustrated by the work of Izumchenko et al. It is our hope that precision medicine, based on improvements in risk assessment and pharmacologic targeting of relevant, high-risk molecular events will become reality as part of the management of OPMLs in the near future.

Conflicts of interest

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References


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