Published OnlineFirst February 24, 2015; DOI: 10.1158/1940-6207.CAPR-15-0057

Perspective

Cancer Prevention Research

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

William N. William Jr[1] and Adel K. El-Naggar[2]

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. A similar molecular characterization of potentially malignant oral epithelial lesions (OPML; 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 and colleagues 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. *Cancer Prev Res; 8(4); 262–5. ©2015 AACR.*

See related article by Izumchenko et al., p. 277

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 (OPML) 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 population-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).

[1]Department of Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas. [2]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Corresponding Author: William N. William Jr, Department of Thoracic/Head and Neck Medical Oncology, Division of Cancer Medicine, 1515 Holcombe Boulevard, Unit 432, The University of Texas MD Anderson Cancer Center, Houston, TX 77030. Phone: 713-792-6363; Fax: 713-792-1220; E-mail: wnwillia@mdanderson.org

doi: 10.1158/1940-6207.CAPR-15-0057

The underlying molecular events of oral carcinogenesis have been the subject of intense investigation. In 1996, Califano and colleagues (4) proposed a genetic progression model of oral cancers by analyzing 10 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 histologic 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 the 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 and 9p21). Based on the high frequency of LOH in invasive cancers, Mao and colleagues (6) reported the incidence and the prognostic significance of LOH at 3p14 and 9p21 in oral leukoplakia. The

Published OnlineFirst February 24, 2015; DOI: 10.1158/1940-6207.CAPR-15-0057

authors demonstrated that LOH at these loci was associated with a 5-fold increase in the risk of invasive cancer development. 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 whether 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 (i) are fully characterized preclinically and clinically, in regard to their role in oral carcinogenesis, and (ii) 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) have 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*. The use of smokeless tobacco was nearly universally present in this cohort, which could explain some of the differences compared with previous reports (15). Likewise, Song and colleagues (16) recently reported a frequency of 43% of nonsynonymous *NOTCH1* somatic mutations in 51 Chinese patients. In 36% of the patients with mutated tumors, the *NOTCH1* mutations were localized to the heterodimerization domain, where most of the activating mutations in hematologic malignancies occur, in sharp contrast to the *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 *Cancer Prevention Research*, Izumchenko and colleagues (17) report on the prevalence of *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 *NOTCH1* mutation. Consistent with the findings previously described by Song and colleagues (16), a substantial proportion of the *NOTCH1* mutations identified in this defined ethnic cohort were postulated to confer gain of function. The authors concluded that *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 the 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 to move forward with NOTCH1-based strategies to either stratify and/or treat OPML patients within the context of chemoprevention studies; the mere presence of *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 are 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 premalignancies.

Despite these limitations, the work by Izumchenko and colleagues 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 gastrointestinal 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

Published OnlineFirst February 24, 2015; DOI: 10.1158/1940-6207.CAPR-15-0057

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 and colleagues 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 multiplatform 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 and colleagues. 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.

Disclosure of Potential Conflicts of Interest

W.N. William Jr has received research support from Astellas. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: W.N. William Jr, A.K. El-Naggar
Development of methodology: W.N. William Jr
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.N. William Jr
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W.N. William Jr
Writing, review, and/or revision of the manuscript: W.N. William Jr, A.K. El-Naggar
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W.N. William Jr
Study supervision: W.N. William Jr

Grant Support

This work was supported by the NCI grants P30 CA016672-36 (to Ronald DePinho), P50 CA097007-09, and by the Cancer Prevention and Research Institute of Texas grant RP140464 (to William N. William Jr.).

Received February 11, 2015; accepted February 17, 2015; published OnlineFirst February 24, 2015.

References

1. Gupta PC, Mehta FS, Daftary DK, Pindborg JJ, Bhonsle RB, Jalnawalla PN, et al. Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. Community Dent Oral Epidemiol 1980;8:283–333.
2. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer 1984; 53:563–8.
3. William WN Jr. Oral premalignant lesions: any progress with systemic therapies? Curr Opin Oncol 2012;24:205–10.
4. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996;56:2488–92.
5. Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, et al. A transcriptional progression model for head and neck cancer. Clin Cancer Res 2003;9:3058–64.
6. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med 1996;2:682–5.
7. Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res 2000;6:357–62.
8. Rosin MP, Lam WL, Poh C, Le ND, Li RJ, Zeng T, et al. 3p14 and 9p21 loss is a simple tool for predicting second oral malignancy at previously treated oral cancer sites. Cancer Res 2002;62:6447–50.
9. Zhang L, Poh CF, Williams M, Laronde DM, Berean K, Gardner PJ, et al. Loss of heterozygosity (LOH) profiles—validated risk predictors for progression to oral cancer. Cancer Prev Res 2012;5:1081–9.
10. William WN, Papadimitrakopoulou VA, Lee JJ, Mao L, Lin HY, Gillenwater AM, et al. Randomized placebo-controlled trial (RCT) of erlotinib for prevention of oral cancer (EPOC). J Clin Oncol 32:5s, 2014 (suppl; abstr 6007).
11. Taoudi Benchekroun M, Saintigny P, Thomas SM, El-Naggar AK, Papadimitrakopoulou V, Ren H, et al. Epidermal growth factor receptor expression and gene copy number in the risk of oral cancer. Cancer Prev Res 2010; 3:800–9.
12. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 2011;333:1154–7.
13. Hayes DN, Grandis JR, El-Naggar AK. The Cancer Genome Atlas: integrated analysis of genome alterations in squamous cell carcinoma of the head and neck. J Clin Oncol 31, 2013 (suppl; abstr 6009).
14. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science 2011;333:1157–60.
15. Maitra A, Biswas NK, Amin K, Kowtal P, Kumar S, Das S. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. Nat Commun 2013;4:2873.
16. Song X, Xia R, Li J, Long Z, Ren H, Chen W, et al. Common and complex Notch1 mutations in Chinese oral squamous cell carcinoma. Clin Cancer Res 2014;20:701–10.
17. Izumchenko E, Sun K, Jones S, Brait M, Agrawal N, Koch WM, et al. Notch1 mutations are drivers of oral tumorigenesis. Cancer Prev Res 2015;8: 277–86.
18. Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269–71.
19. Villamor N, Conde L, Martinez-Trillos A, Cazorla M, Navarro A, Beà S, et al. NOTCH1 mutations identify a genetic subgroup of chronic lymphocytic

Published OnlineFirst February 24, 2015; DOI: 10.1158/1940-6207.CAPR-15-0057

leukemia patients with high risk of transformation and poor outcome. Leukemia 2013;27:1100–6.
20. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, Woolcock B, et al. Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood 2012;119:1963–71.
21. Wu Y, Cain-Hom C, Choy L, Hagenbeek TJ, de Leon GP, Chen Y, et al. Therapeutic antibody targeting of individual Notch receptors. Nature 2010;464:1052–7.
22. Rosati E, Sabatini R, Rampino G, Tabilio A, Di Ianni M, Fettucciari K, et al. Constitutively activated Notch signaling is involved in survival and apoptosis resistance of B-CLL cells. Blood 2009;113:856–65.
23. Andersson ER, Lendahl U. Therapeutic modulation of Notch signalling—are we there yet? Nat Rev Drug Discov 2014;13:357–78.
24. Hernandez Tejada FN, Galvez Silva JR, Zweidler-McKay PA. The challenge of targeting notch in hematologic malignancies. Front Pediatr 2014;2:54.

Published OnlineFirst February 24, 2015; DOI: 10.1158/1940-6207.CAPR-15-0057

Cancer Prevention Research

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

William N. William, Jr and Adel K. El-Naggar

Cancer Prev Res 2015;8:262-265. Published OnlineFirst February 24, 2015.