Editorial

Cancer Prevention Research

When the Damage Is Done: Selecting Patients for Head and Neck Cancer Chemoprevention Trials

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See related article by Khariwala et al., p. 507

Among the 600,000 cases of head and neck squamous cell carcinoma (HNSCC) expected worldwide in 2017, the substantial majority is caused by environmental rather than viral carcinogenesis (1). Half of attributable environmental risk can be classified as proximal, where individual behavior results in direct carcinogen exposure, such as to tobacco, alcohol, or betel quid. However, half of attributable risk is distal, where carcinogen exposure is unavoidable due to second-hand smoke, industrial pollution, pestilence, or war (2). Because of compelling epidemiology linking proximal exposure to outcome, HNSCC has been a major target of the chemoprevention field over the past 50 years. Despite randomized clinical trials evaluating multiple promising compounds, including isotretinoin, celecoxib, and erlotinib, no agent has proven effective and tolerable for prevention of HNSCC (3). A significant barrier to efficient chemoprevention trials has been the dearth of biomarkers to individualize risk and select patients with a high enough HNSCC event rate to justify intervention, particularly among otherwise healthy smokers (4). In this issue of *Cancer Prevention Research*, Khariwala and colleagues present a novel and mechanistically relevant biomarker that may overcome this barrier. In a case–control study comparing current smokers with HNSCC with smokers without cancer, tobacco carcinogen-specific DNA damage quantified in buccal cells was an independent risk factor for HNSCC. These findings point to a high-risk population that would benefit from targeted tobacco cessation programs and increased surveillance. Equally important, this damage biomarker could provide an innovative selection strategy for primary chemoprevention trials.

"Number needed to treat" (NNT) is a measure of effectiveness of an intervention, and in chemoprevention describes the number of patients who must be treated to prevent a single case of HNSCC. As NNT is inversely related to disease incidence and effectiveness of the intervention, primary prevention trials for HNSCC are prohibitively large. Thus, clinical trials have focused on two secondary prevention populations: (i) those with an oral premalignant lesion (OPL), where risk of HNSCC is 11% to 70% over 30 years; (ii) patients curatively treated for a first environmental HNSCC, who develop second primary tumors (SPT) at the rate of 3% to 6% per year (3). The multistep transformation of oral epithelial cells is initiated by DNA damage and illustrated by the histologic spectrum of OPLs, including hyperplasia, dysplasia, and carcinoma *in situ*. Historically, proof-of-concept chemoprevention trials in HNSCC have assessed a compound's ability to reverse OPLs, which are associated with carcinogen exposure and can be clinically identified as leukoplakia. Although this strategy permits selection of patients with a higher risk of HNSCC than a smoking population without lesions, such trials are difficult to accrue as OPLs are relatively rare. Moreover, OPLs are an unreliable marker of risk, often failing to progress and even undergoing spontaneous regression in approximately 30% of cases. To date, the best molecular biomarker of HNSCC risk is loss of heterozygosity (LOH) of the 3p and 9p chromosomal regions within a resected OPL, where patients have a 35% risk of HNSCC within 3 years. Although LOH was prospectively validated as a prognostic biomarker in the EPOC trial investigating erlotinib (5), this biomarker has limited applicability due to the requirement for an OPL that harbors LOH, parsing an already rare population. Quantitative determination of buccal cell DNA damage, as presented by Khariwala and colleagues, represents a biomarker from histologically normal mucosa and, thus, could be broadly applied to the entire smoking population, raising the promise of feasible primary prevention trials.

N′-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are key nitrosamine carcinogens found in tobacco products. Urinary levels of NNN and NNK metabolites provide an indication of tobacco exposure and have been linked to development of esophageal and lung carcinomas (6). Metabolism of NNN and NNK also results in DNA adduct formation, and acid hydrolysis of these adducts leads to release of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). In an earlier study, Ma and colleagues devised a highly sensitive liquid chromatography nanoelectrospray–high-resolution tandem mass spectrometry method to detect HPB released from buccal cell DNA (7). Using this assay, Khariwala and colleagues compared levels of buccal DNA adducts, controlling for NNN and NNK urinary metabolites, in 35 cancer-free smokers versus 30 smokers with HNSCC. Analysis of urinary metabolites revealed a similar level of exposure to tobacco carcinogens in the two groups. In contrast, a 20-fold higher level of buccal cell DNA adducts was observed in smokers with HNSCC when compared with cancer-free smokers after adjusting for modest demographic differences. This represents the first demonstration of elevated DNA adducts in "normal" tissue from cancer versus cancer-free smoking subjects. Notably, adduct levels were measured in buccal cells acquired noninvasively, by brushing the inner check.

Although buccal DNA adduct levels represent a promising biomarker of HNSCC risk, findings from a small case–control study require stepwise, prospective validation as case–control methodology is particularly prone to selection bias. Despite appropriate multivariate adjustment, results may also be confounded by unmeasured exposures to other tobacco carcinogens and host DNA repair capacity. Potential validation steps include measurement of buccal cell DNA adducts in otherwise healthy, smoking cohorts, to determine association with the

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development of HNSCC. In addition, the relationship between buccal DNA adducts and SPT development could be studied in patients treated for a first tobacco-related HNSCC. Extending these studies beyond smokers would also be impactful, as chronic exposure to second-hand smoke is often not voluntary and represents a risk factor for development of upper aerodigestive tract cancers. Unfortunately, the current methodology is specific to tobacco nitrosamines and is not applicable to people with proximal exposure to betel quid, a practice deeply ingrained in many Asian cultures and affecting approximately 600 million people worldwide. However, betel quid contains carcinogenic nitrosamines derived from the areca nut that may be mechanistically similar to NNN and NNK and potentially amenable to assays of DNA adduct formation (8). As this methodology requires ongoing exposure to and metabolism of tobacco-specific nitrosamine carcinogens, it is unlikely to be useful as a marker of "condemned epithelium" in the context of people who have quit tobacco use. Moreover, the measurement of tobacco carcinogen-specific DNA adducts cannot quantify the burden of distal exposure to industrial carcinogens, many of which overlap with tobacco, including polycyclic aromatic hydrocarbons, aldehydes, phenols, and volatile hydrocarbons.

The mechanism(s) responsible for elevated DNA damage in certain smokers remains unclear. As noted by the authors, carcinogen-induced DNA damage is modulated by several factors, including carcinogen exposure, metabolic activation, excretion, and DNA repair capacity. Although not statistically significant, greater CYP2A6 activity was detected in smokers with HNSCC. Because CYP2A6 plays an important role in both the metabolism of nicotine and the metabolic activation of NNN and NNK (9), these results may be biologically significant. Buccal DNA adduct levels likely reflect a balance between metabolic activation and DNA repair. Thus, in addition to performing CYP2A6 genotyping in this cohort, a future analysis proposed by the authors, consideration should be given to evaluating SNPs among enzymes responsible for repair of NNN and NNK-induced DNA adducts, including O^6-alkylguanine DNA alkyltransferase, XRCC1, and ERCC2 (10). Such studies form an important piece of mechanistic evidence and could suggest that high levels of buccal DNA adducts in fact are part of a causal chain. If this is the case, this biomarker has the potential to be not only prognostic, but predictive, in the setting of a chemopreventive agent purported to enhance DNA repair capacity.

Environmental HNSCC is both morbid and lethal, with a 5-year overall survival of 40% to 50%. Although elimination of carcinogen exposure remains the top priority for HNSCC prevention, this frequently is not achievable due to a complex matrix of barriers including addiction biology, cultural norms, and socioeconomic barriers. The worldwide association of both proximal and distal carcinogen exposures with low socioeconomic status means that environmental HNSCC is frequently a disease of the poor and dispossessed. Moreover, exposures can be compounded by reduced access to diets rich in phytochemicals thought to mitigate DNA damage from carcinogens. The development of an effective and affordable chemoprevention strategy for HNSCC represents a major unmet global need. To date, this effort has been hindered by a low incidence rate in the at-risk population and lack of efficacy of candidate agents, making the NNT prohibitive and precluding efficient clinical trials. The identification of buccal cell DNA adducts as a potential biomarker of risk suggests that a prevalent high-risk group could be prospectively selected: individuals with unabated exposure to tobacco carcinogens and elevated levels of buccal DNA adducts. Future chemoprevention trials in HNSCC could enroll this target population to hasten the evaluation of novel agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D.E. Johnson, J.E. Bauman
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.E. Johnson, J.E. Bauman
Writing, review, and/or revision of the manuscript: D.E. Johnson, J.E. Bauman
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.E. Johnson, J.E. Bauman
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