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Exfoliated buccal and microdissected lung cell expression of antioxidant enzymes

Shalini V. Kumar MS[a], Ritu Jain PhD[a], Katherine Mokhiber RN[b], Ann Venezia RN[b], Angela Sheehan MS[b], Simon D. Spivack MD[a,b,c,*]

[a] *Laboratory of Human Toxicology and Molecular Epidemiology, Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509, USA*

[b] *Albany Medical College, Pulmonary and Critical Care Medicine, 47 New Scotland Avenue, Albany, NY 12208, USA*

[c] *SUNY School of Public Health, Environmental Health and Toxicology, Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509, USA*

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Abstract

Introduction: An exfoliated buccal cell biomarker assay for antioxidant gene transcript levels was used to measure inter-tissue concordance with lung, and inter-subject variability in a lung cancer case-control study. *Methods:* First, *qualitative* RNA-specific RT-PCR was used to compare expression in exfoliated buccal cells with that in laser microdissected lung tissue remote from the tumor from 14 individuals providing both specimens. *Results:* There was complete [100% for quinone oxidoreductase 1 (NQO1), glutathione peroxidase (GPX), and superoxide dismutase 1 (SOD1)], or predominant [85.7% for catalase (CAT)] inter-tissue concordance for qualitative expression. Second, *quantitative* real-time RT-PCR for antioxidant enzyme transcript levels was performed in exfoliated buccal samples from these same 14 individuals, as well as 28 additional individuals providing buccal cells only, for a total of 42 buccal specimens (19 current smokers and 23 ex- or never-smokers), of whom 26 (61.39%) had a new diagnosis of lung cancer. *Discussion:* Wide inter-individual expression differences for each gene transcript ($>10^1$–10^4-fold) were observed in the exfoliated buccal cells, unrelated to smoking and case-control status. In multivariate analyses, family history of tobacco-related malignancy correlated inversely with buccal NQO1 and CAT mRNA levels ($p = 0.003$, $p < 0.001$, respectively). This antioxidant expression trait may relate to family risk of cancer, but is notably unrelated to oxidant challenges inherent in cigarette smoke.

Keywords: Gene expression; Antioxidant metabolism; Biomarkers; Genetic susceptibility; Exfoliated cells; Familial risk of cancer; Smokers; Never smokers; Inter-tissue concordance; Lung cancer case-control study; Tobacco-related expression; Catalase; Glutathione peroxidase; Plasma nicotine; Plasma cotinine; Diet; Reverse transcriptase protocol

1. Introduction

The scientific effort to identify non-invasive exposure or disease biomarkers is aimed at a determining: functional internal dose of a foreign compound; the proximate biological effect of that exposure; intermediate disease processes; for diagnostics of overt disease, and detection of therapeutic toxicity. The use of exfoliated, easily accessible epithelial samples amenable to such analyses constitutes an important approach to cancer prevention research.

Buccal mucosal cells are epithelial in origin, are exposed to inhaled and ingested environmental toxicants directly through mucosal contact and via the circulation, and are easily accessible for analysis. Exfoliated buccal cells have been used as sources for genomic DNA in a variety of settings [1–4]. Buccal cell gene expression has been documented in surgical specimens, explants, and primary cultures of malignant cells [5–7], and this laboratory recently reported quantitative gene expression studies using brushed exfoliated cytologic buccal specimens, for the determination of carcinogen metabolizing enzyme transcript levels [8].

Oxidative challenge to the human airway is common under physiologic conditions, because oxygen is present at

* Corresponding author.
E-mail address: spivack@wadsworth.org (S.D. Spivack).

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high partial pressures, and because free radicals are a common by-product of general, and particularly xenobiotic and mitochondrial metabolism. In the lung, under pathophysiologic conditions of general inflammation and injury, in chronic lung diseases such as chronic bronchitis, emphysema, and fibrosing disorders, free radical burden derived from a variety of cell types is dramatically increased. The inhalation of tobacco smoke-derived free radicals in the form of lipid peroxides adds to the burden of oxidants derived from inflammation [9]. In carcinogenesis, both initiation, via mutation from oxidation of DNA bases, and promotion, via increased cell turnover, have been associated with oxidative processes. Cigarette smoke has been shown to cause DNA damage in cultured human lung cells [10] and reactive oxygen species (ROS) such as H_2O_2 and $O_2^{\bullet -}$ are known to be partly responsible of this damage [11].

Defense against ROS resides, in part, with known antioxidant enzymes (AOE), including cytosolic copper zinc superoxide dismutase (SOD1), catalase (CAT), and glutathione peroxidase (GPX). These enzymes have been clearly demonstrated to be up-regulated in animal and human models under conditions of oxidative stress [12–14]. The frequency of oxidative DNA damage, as measured by gas chromatography-mass spectrometry (GC-MS), may be inversely proportional to AOE activity in pulverized human lung tumor tissue, and the highest AOE levels have been found in surrounding non-tumor tissue, among smokers [15–17]. There are suggestions of a direct or indirect biological coupling of the xenobiotic metabolism and antioxidant pathways [14,18,19]. NADPH-dependent quinone oxidoreductase (NQO1) is an oxidant-quenching enzyme, catalyzing the two-electron reduction of quinones, quiinone amines, and azo dyes. This enzyme's activity appears to be regulated at the transcriptional level, with an aromatic hydrocarbon receptor (Ahr)-binding xenobiotic response element (XRE) motif being one of the main regulatory factors in experimental studies [20]. Wide inter-individual variability in antioxidant gene expression in cells brushed from the lower airway epithelium has been reported [12].

We hypothesize that quantitative AOE gene expression levels influenced by tobacco smoke exposure are relevant to airway risk for malignancy. Both expression phenotypes and exposure require precise measurement, if one is to assess the gene-environment interplay with accuracy. In this study, we report exfoliated buccal cell transcript expression for four of the classical AOEs: NQO1, CAT; GPX, and SOD1, in the context of chemically determined levels of tobacco exposure.

2. Materials and methods

2.1. Human subjects

2.1.1. Subjects

All procedures were performed with approval of institutional review boards at Albany Medical Center and the New York State Department of Health. Recruits were part of the pulmonary medicine or thoracic surgery practices at Albany Medical Center, and were otherwise destined for bronchoscopy or lung resectional surgery for clinical indications, as previously described [8,21]. After informed consent was obtained, each subject was interviewed, his/her buccal mucosa was sampled by cytologic brush, and the subject's blood was drawn at the same encounter, in advance of the lung-sampling procedure. Pathologic diagnoses on lung tissue were available for 40 of 42 subjects; two of 42 individuals (both controls) were categorized on clear, unambiguous clinical grounds alone. In aggregate, 26 subjects providing buccal cells were lung cancer cases, and 16 were lung cancer controls. The subset of 14 consecutive subjects donating lung tissue for gene expression research purposes (10 lung cancer cases and four non-cancer controls; four current smokers and 10 former or ex-smokers), were selected based on lung tissue availability. All lung cancer diagnoses were new (incident) bronchogenic carcinoma diagnoses, at any clinical stage; enrollment, interview, and biospecimen collection preceded any therapeutic interventions. Non-malignant lung disease (COPD, emphysema, chronic bronchitis, interstitial lung disease) were self-reported, and compared with the medication list for consistency.

2.1.2. Interview data collected

Subjects offered information on mainstream tobacco smoking history (type, amount, duration, quitting date); environmental tobacco smoke exposure history; pre-existing non-malignant lung diagnoses (COPD, interstitial lung disease); dietary history (daily average ingested servings of fruits and vegetables and cups of tea, supplements, restrictions); asbestos- and radon-exposure history; occupational history; family history of tobacco-related (defined as lung, head and neck, bladder, stomach, pancreas) and all cancer; and medication history. These data are summarized in Table 1. Precise current tobacco exposure was verified by assay of plasma nicotine and cotinine, in the Analytic Chemistry Laboratory at Wadsworth Center, as described below. Characteristics of the 42 subjects, categorized by historical smoking status, are described in Table 1.

2.2. Cytologic buccal epithelium specimen collection

Buccal biospecimen sampling by cytologic brush was performed by trained research nurses at a tertiary care center (Albany Medical Center), as previously described [8]. A cytologic brush (e.g. Cytobrush® Plus GT; Medscand Inc., Hollywood, FL) with tapering plastic bristles and a blunt end was placed in the subject's mouth. Sufficient lateral pressure was applied so as to contact the buccal mucosa to cause slight bending of the plastic shaft. The brush was spun in place for 10 s, in a single direction, while consistent pressure was applied to the mucosa. There were no significant episodes of irritation or bleeding. The brush was then withdrawn and immediately plunged into the RNA