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Tobacco smoking-response genes in blood and buccal cells

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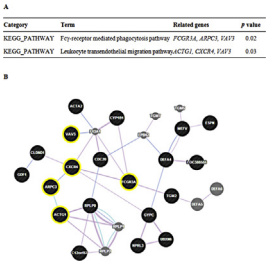
HIGHLIGHTS

- A biological monitoring was performed to find tobacco smoking-specific toxic mechanisms in surrogate and non-invasive tissues.
- The Fcγ-receptor mediated phagocytosis and leukocyte transendothelial migration pathways were differentially expressed between smokers and nonsmokers.
- The *ACTG1*, involved in the maintenance of actin cytoskeleton, cell migration and cancer metastasis, was highly expressed in both of blood and buccal cells.
- Smokers showed high levels of urinary malondialdehyde (MDA) and down-regulation of expressions of antioxidant related genes including *TPO*, *MPO*, *GPX2*, *PTGR1*, and *NUDT1*.

GRAPHICAL ABSTRACT



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ABSTRACT

Tobacco smoking is a well-known cause of various diseases, however, its toxic mechanisms for diseases are not completely understood, yet. Therefore, we performed biological monitoring to find tobacco smoking-responsive mechanisms including oxidative stress in Korean men ($N = 36$). Whole genome microarray analyses were performed with peripheral blood from smokers and age-matched nonsmokers. We also performed qRT-PCR to confirm the microarray results and compared the gene expression of blood to those of buccal cells. To assess the effects of tobacco smoking on oxidative stress, we analyzed urinary levels of malondialdehyde (MDA), a lipid peroxidation marker, and performed PCR-based arrays on reactive oxygen species (ROS)-related genes. As results, 34 genes were differently expressed in blood between smokers and nonsmokers ($ps < 0.01$ and >1.5-fold change). Particularly, the genes involved in immune responsive pathways, e.g., the Fcγ-receptor mediated phagocytosis and the leukocyte transendothelial migration pathways, were differentially expressed between smokers and nonsmokers. Among the above genes, the *ACTG1*, involved in the maintenance of actin cytoskeleton, cell migration and cancer metastasis, was highly expressed by smoking in both blood and buccal cells. Concerning oxidative

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stress, smokers showed high levels of urinary MDA and down-regulation of expressions of antioxidant related genes including *TPO*, *MPO*, *GPX2*, *PTGR1*, and *NUDT1* as compared to nonsmokers ($ps < 0.05$). In conclusion, these results suggest that systemically altered immune response and oxidative stress can be tobacco-responsive mechanisms for the related diseases. Based on consistent results in blood and buccal cells, expression of the *ACTG1* can be a tobacco smoking-responsive biomarker.

1. Introduction

Tobacco smoking is the leading avoidable cause of morbidity and mortality worldwide, contributing to approximately 6 million deaths per year (WHO, 2011): it is a major risk factor for cardiovascular diseases (Prasad et al., 2009), airway inflammatory diseases (Arnson et al., 2010) and many types of cancer (Humans, 2012; Jemal et al., 2011).

Toxic components in tobacco or tobacco smoking-induced chemicals, e.g., benzene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and polycyclic aromatic hydrocarbons (PAHs), have been emphasized as causes of tobacco-related toxicity. In addition, bioproduced reactive oxygen species (ROS) by tobacco smoking have been suspected to play a significant role as tobacco-related toxic mechanisms (Yao and Rahman, 2011). However, specific mechanisms of tobacco smoking-related diseases are not completely understood yet (Steiling et al., 2009).

The health risks of tobacco smoking are highly complicated, because tobacco contains more than 4000 chemicals (Wogan et al., 2004), which induce a variety of physiological responses. For example, many constituents of tobacco, such as nicotine, benzo[a] pyrene, and hydroquinone, have been reported to induce immune alteration (Stampfli and Anderson, 2009). Therefore, we decided to integrate scattered and complicated toxic information of tobacco smoking for clear understanding of its toxic mechanisms. For this purpose, genome-wide expression profiling has emerged as a powerful approach for identification of molecular responsive markers, prediction and relation to clinical outcome of tobacco smoking (Gower et al., 2011). Previous genome wide studies suggested that tobacco smoking altered pathways involved in regulation of oxidative stress (Wang et al., 2010), carcinogenesis (Charles et al., 2008), or host defense (Doyle et al., 2010) with microarray based gene expression profiling. However, these results are not consistent. It may be due to multiple reasons: first, ethnic variations, e.g., in Caucasians, Asians, or Hispanics, etc., may be one of causes of inconsistent results (Boyle et al., 2010; Charlesworth et al., 2010; Shahdoust et al., 2013). Secondly, lack of accurate biological monitoring of tobacco smoking may be a reason. Thirdly, the different types of tissues, e.g., blood, buccal, or bronchial epithelium, may generate inconsistent results (Beineke et al., 2012; Boyle et al., 2010; Wang et al., 2010). In order to identify reliable targets of tobacco smoking, surrogate biospecimen or multiple tissues are needed.

In the present study, we performed whole genome expression analyses between Korean male smokers and nonsmokers to understand tobacco smoke-related pathological mechanisms and clarify the gene expression profiles in surrogate and noninvasive tissues. At first, we performed whole genome microarrays to estimate the systemic influence of tobacco smoking. Based on the microarray results, we confirmed microarray results on selected genes in both blood and buccal epithelium cells to evaluate their appropriateness as responsive or effective biomarkers for tobacco smoking. To further investigate the effect of tobacco smoking on the oxidative stress response pathway, we analyzed tobacco smoking-induced expression profiles of ROS-related genes and urinary malondialdehyde (MDA), a typical biomarker for oxidative stress.

2. Materials and methods

2.1. Study subjects

We recruited 36 healthy Korean men (age = 40.2 ± 5.9 years) at Eulji University Hospital (Daejeon, South Korea). The study scheme is shown in Fig. 1. All subjects provided a written informed consent and completed extensive questionnaires including medical and smoking history, dietary patterns, alcohol drinking, environment of residency, etc. Carbon monoxide (CO) in blood was analyzed in all the subjects with MicroCO breath CO Monitor (CareFusion, San Diego, CA). All of the study protocol was approved by Institutional Review Board of Eulji University Hospital.

2.2. Sample collection

We collected buccal cell samples from the subjects with sterile cotton swabs: the subjects rinsed their mouth twice with 200 ml of drinking water and the swabs were scraped gently more than 10 times against the buccal mucosa on the inside of the cheek. The cells in swab head were immediately immersed in 500 μl of RNA-later® stabilization reagent (QIAGEN, Valencia, CA) and stored at −80 °C until analyses. We also collected 40 ml of urine and 10 ml of peripheral blood into 50 ml of conical tubes and PAXgene™ Blood RNA tubes (QIAGEN), respectively, and stored them at −80 °C until analyses.

2.3. Analyses of urinary cotinine

Urinary cotinine was analyzed by ion-pair HPLC/UVD method (Yang et al., 2001) with minor modifications. In brief, 900 μl of each urine sample was mixed with 100 μl of 80 μM 2-phenylimidazole, as an internal standard, and 330 μl of 3 M NaOH. The mixture was twice extracted with 3 ml of CH_2Cl_2 per each. After evaporating CH_2Cl_2-extract, we dissolved the residue in 1 ml of water and injected 20 μl of its supernatant fraction to HPLC. The HPLC system consisted of dual Younglin SP930D pumps (Younglin, Seoul, Korea), the MIDAS COOL auto sampler (Spark Holland, Emme, The Netherlands), the SPD-10A UV–vis detector (Shimadzu, Kyoto, Japan), and the TSK gel ODS-80™ column (5 μm, 4.6 mm × 150 mm, Toyo Soda Co., Toyko, Japan). The analyses were carried out with the gradient mode: mobile phase A, a mixture of acetonitrile/water (15/85) containing 20 mM KH_2PO_4 and 3 mM sodium 1-octanesulfonate (pH 4.5); B, methanol; flow rate was 0.7 ml/min, ratio of A to B, 0–20 min, 100:0; 20–25 min, 100:0 to 50:50; 25–30 min, 50:50; 30–35 min, 50:50 to 100:0; 35–45 min, 100:0. Column was kept at 50 °C and the absorbance was observed at 254 nm.

Urinary cotinine was adjusted for urinary creatinine, measured with ion-pair HPLC/UVD method, as described by Ogata and Taguchi (Ogata and Taguchi, 1987) with minor modifications (Yi et al., 2011).