Identification of Biomarkers of Arsenic Exposure and Metabolism in Urine Using SELDI Technology

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Arsenic, a naturally occurring element, is a known human carcinogen. It is a known cause of lung cancer via inhalation and skin cancer via ingestion. Recently IARC classified arsenic in drinking water as a human carcinogen including lung and bladder cancer as outcomes. To obtain mechanistic insight into arsenic toxicity and to identify novel biomarkers of exposure and early biologic effect, we examined the impact of arsenic exposure on the human proteome in a study of individuals exposed to arsenic in their drinking water.

All subjects participated in an earlier study of micronuclei in exfoliated bladder cells to evaluate the possible genotoxic effects of chronic arsenic ingestion on the bladder. The study was conducted in Churchill County, NV, in a location where private wells were sometimes contaminated with high concentrations of arsenic. Because arsenic is thought to cause genotoxic damage to the bladder, we used urine from exposed and unexposed individuals to identify biomarkers of arsenic exposure and early biologic effect using SELDI technology. Exposed individuals had well characterized exposures ($N = 18$; average well water As concentration 1312 ug/L ±359). They were matched by age, gender, and smoking status to low-exposed controls ($N = 18$; average well water As concentration 16 ug/L ±7). First morning urine voids were collected and stored at -80°C until they were used to measure urinary arsenic concentration of inorganic arsenic and its methylated metabolites. Samples were thawed once before being thawed for proteomic analysis. Urine samples were subjected to anion exchange fractionation and bound to three types of protein chips (CM10, IMAC-30, and NP20). Protein expression patterns were detected by SELDI-TOF MS. Each sample was run in duplicate, and an internal control sample randomly added to each chip to determine interchip variability. Blank samples were also run as negative controls.

Signal intensity was low in these samples because of the overall low abundance of protein in urine. The lower abundance and the lower peak heights also increased sample variability. Based on a pooled coefficient of variance (CV pooled), the variance was highest for the NP20 chip and lowest for the CM10 chip surface. The CM10 chip also had more peaks of higher intensity than the other two chip types. Up- and down-regulation of several proteins was observed in samples from arsenic exposed subjects. Analysis of these data is ongoing.

This study demonstrates the potential of the urine proteome as a noninvasive means to identify biomarkers of exposure and metabolism to toxic chemicals. SELDI-TOF analysis of a small number of highly exposed and unexposed subjects revealed altered expression of several proteins that can be identified as intermediate biomarkers of early effect. We are planning future studies to confirm this work using urine samples from other exposed populations using a larger number of samples from exposed and unexposed individuals.

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