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Short communication

Proteomic profiling reveals candidate markers for arsenic-induced skin keratosis☆

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ABSTRACT

Proteomics technology is an attractive biomarker candidate discovery tool that can be applied to study large sets of biological molecules. To identify novel biomarkers and molecular targets in arsenic-induced skin lesions, we have determined the protein profile of arsenic-affected human epidermal stratum corneum by shotgun proteomics. Samples of palm and foot sole from healthy subjects were analyzed, demonstrating similar protein patterns in palm and sole. Samples were collected from the palms of subjects with arsenic keratosis (lesional and adjacent non-lesional samples) and arsenic-exposed subjects without lesions (normal). Samples from non-exposed healthy individuals served as controls. We found that three proteins in arsenic-exposed lesional epidermis were consistently distinguishably expressed from the unaffected epidermis. One of these proteins, the cadherin-like transmembrane glycoprotein, desmoglein 1 (DSG1) was suppressed. Down-regulation of DSG1 may lead to reduced cell-cell adhesion, resulting in abnormal epidermal differentiation. The expression of keratin 6c (KRT6C) and fatty acid binding protein 5 (FABP5) were significantly increased. FABP5 is an intracellular lipid chaperone that plays an essential role in fatty acid metabolism in human skin. This raises a possibility that overexpression of FABP5 may affect the proliferation or differentiation of keratinocytes by altering lipid metabolism. KRT6C is a constituent of the cytoskeleton that maintains epidermal integrity and cohesion. Abnormal expression of KRT6C may affect its structural role in the epidermis. Our findings suggest an important approach for future studies of arsenic-mediated toxicity and skin cancer, where certain proteins may represent useful biomarkers of early diagnoses in high-risk populations and hopefully new treatment targets. Further studies are required to understand the biological role of these markers in skin pathogenesis from arsenic exposure.

1. Introduction

Arsenic, one of the most toxic metalloids, is widely encountered in the environment arising naturally and from pharmaceutical, agricultural and industrial applications (Mead, 2005) that can appear in groundwater. Globally, millions of people are suffering from exposure to hazardous concentrations of arsenic in their water supplies (Wang et al., 2002). Arsenic-induced skin lesions are endemic in many remote mountainous regions in China, especially in the Shanyin district (Guo et al., 2003). Average arsenic concentrations in groundwater there are nearly 17 times higher than the United States Environmental Protection Agency guideline of 10 μg/L, and typical clinical features of hyperkeratosis of palms and soles are observed (Cui et al., 2013). Previous work has found that individuals in this region had higher arsenic content in hair and nail than the average Chinese, consistent with arsenic induction of dermatological lesions (Cui et al., 2013). Those people with noncancerous skin lesions are at high risk of developing skin and internal cancers (Mead, 2005). Epidemiologic studies have revealed that chronic arsenic exposure can induce cancer and a host of adverse health effects in the digestive, respiratory, cardiovascular and nervous systems (Matschullat, 2000).

Possible mechanisms of arsenic action have been proposed,

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including arsenic-induced oxidative stress, chromosomal damage, altered transcription factor levels, and impaired DNA repair (Yu et al., 2006). However, further details of arsenic's mechanism of action still need to be elucidated. Identifying reliable molecular markers will be valuable for understanding the manifestations of arsenic-induced skin disease, a challenge that emerging omics technologies could meet (Vlaanderen et al., 2010). Proteomic analysis can help to identify proteins that are differentially expressed in afflicted tissue, which may reveal useful diagnostic markers or even drug targets. Rodent and cell models have provided valuable information on arsenic action (Tokar and Waalkes, 2011), but studies of human samples are urgently needed. Research on biomarkers of arsenic-exposed human samples, such as skin lesions, is very limited. Previous studies have analyzed arsenic-exposed human serum and urinary proteomic profiles and identified several potential biomarkers (Harezlak et al., 2008; Hegedus et al., 2008; Zhai et al., 2005). According to the IARC, skin shows the strongest association between chronic arsenic exposure and cancer (IARC, 2004), and dermal manifestations such as hyperpigmentation and hyperkeratosis are diagnostic of chronic arsenicosis. Arsenical hyperkeratosis appears predominantly on the palms of the hands and soles of the feet (Liu et al., 2002).

Two-dimensional gel electrophoresis has been applied in arsenic-related proteomic studies (Yu et al., 1993), but this method has the disadvantages of low reproducibility, poor representation of low abundant proteins, inaccurate quantification, high labor and time requirements, and inability to analyze the membrane and hydrophobic proteins (Abdallah et al., 2012). Shotgun proteomics, which utilizes reverse phase separation of peptides instead of electrophoretic protein separation, has higher speed, sensitivity, accuracy, and throughput with lower sample consumption (Zhang et al., 2013). In this study, human palm samples from arsenic-exposed skin-diseased subjects (lesional and non-lesional epidermis), arsenic-exposed normal subjects (normal) and non-exposed healthy subjects (control) were obtained for shotgun proteomic analysis. The response of palm to arsenic was investigated by comparing protein expression profiles of lesional epidermis with unaffected epidermis with the aim of finding alterations associated with arsenic exposure. Protein alterations may be useful biomarkers for early diagnoses and may suggest new treatment targets. In addition, healthy foot epidermal samples were also collected to determine the degree of difference between human palm and foot skin in healthy subjects.

2. Materials and methods

2.1. Study participants

This study was approved by the Institutional Review Board at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. All affected and healthy subjects or their legal guardians provided written and informed consent. A total of 21 individuals (Table S1) were recruited for sampling and were all checked by a physician to ensure the presence or absence of skin disease. Arsenic-exposed diseased palm samples (lesional and adjacent non-lesional samples from the same palm) were collected from six individuals who resided in the arsenic-contaminated area in Shanyin district throughout their lives and had severe skin lesions. Arsenic-exposed unaffected palm samples (normal) were obtained from eight individuals from the same place in the Shanyin district without skin pathologic symptoms. Non-exposed samples (control) were obtained from seven individuals with no prior history of arsenic exposure or any skin disease. For the palm and foot sole comparison, additional foot samples were collected from the normal and control groups.

Stratum corneum from palms and foot soles of subjects were collected by applying a 22-mm diameter tape circle from D-Squame Pro Kits (CuDerm Corp, Dallas, TX) to the skin with circular pressure after fully cleaning the sampling sites (Rice et al., 2013). During the period of sampling, samplers always wore clean disposable gloves and masks. Samples were immediately transferred to sterile 15 mL plastic tubes to minimize any contamination from air. They were incubated in a solution of 2% sodium dodecyl sulfate-0.1 M sodium phosphate (SDS-NaH_2PO_4, pH 7.8) at room temperature for 2 days to detach the cells. Cell pellets were harvested, and re-suspended in 0.4 mL of SDS-phosphate buffer. Protein disulfides were reduced in 25 mM dithioerythritol and alkylated with 50 mM iodoacetamide. Proteins were precipitated with 1 mL of 100% ethanol, rinsed twice with 70% ethanol, and then lysed in fresh 0.1 M ammonium bicarbonate containing 10% acetonitrile and 1% (by weight) TPCK-treated reductively methylated trypsin at room temperature for 3 days. Complete digestion was verified by centrifugation of digests at 1000 rpm for 3 min, with no pellet observed. Samples obtained were adjusted to approximately equal peptide concentration by A280, acidified with 0.1% trifluoroacetic acid, and then electrosprayed into a liquid chromatography tandem mass spectrometer (LC−MS/MS) for further fractionation and identification.

2.2. Protein identification

Sample preparation was carried out as previously reported (Rice et al., 2013). Samples were analyzed by LC-MS/MS using a Thermo-Finnigan LTQ ion trap mass spectrometer coupled with electrospray ionization (ESI) source interfaced with a C18 reverse-phase chromatography column. Samples were run in a block randomized order. Details of sample processing and protein identification were as previously described (Hu et al., 2013).

2.3. Statistical analysis

Spectral counting data were analyzed using JMP Genomics 8.0 software (http://www.jmp.com/en_us/software/jmp-genomics.html, SAS Institute Inc., Cary, NC). The data were imported into JMP Genomics and were log2 transformed with shifting factor as 1. Shifting factor was added back into the data after standardization, avoiding missing values resulting from the data being set to 0 after standardizing data in further log transformation. The log transformed data were normalized by quantile normalization, which normalizes data by aligning ranked columns, computing their mean, and then replacing the original data with the average quantiles. It guarantees identical marginal univariate densities and distribution of each sample (Bolstad et al., 2003). After quantile normalization, significantly differentially expressed proteins for different conditions were identified by ANOVA modeling. The ANOVA model included the subject age, samples from left/right hand, physical condition, exposure years, and sex as fixed effects, and block as random effect. The criteria for selection of significant differential expressed proteins were a fold change >1.5 and a p-value <0.05.

2.4. Bioinformatic analysis

Protein ontology classification was performed by importing the proteins into the protein analysis through evolutionary relationships (PANTHER) classification system (http://www.pantherdb.org/, version 10.0). The proteins were grouped according to their molecular functions, biological processes and protein classes.