P34

Multi-omic and phenotypic reprogramming of oral cells following individual or combined exposures to arsenic and smokeless tobacco

Das S[1]*, Thakur S[1,2], Cahais V[1], Renard C[1], Cuenin C[1], Claeys L[1,3], Cros MP[1], Keïta S[1], Venuti A[1], Sirand C[1], Virard F[1,4], Herceg Z[1], Korenjak M[1], Zavadil J[1]

[1] Epigenomics and Mechanisms Branch, International Agency for Research on Cancer, Lyon, France
[2] Faculty of Science, Charles University, Prague, Czech Republic
[3] Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium
[4]University Claude Bernard Lyon 1, INSERM U1052–CNRS UMR5286, Cancer Research Center, Centre Léon Bérard, Lyon, France
** dass@iarc.who.int*

Ground water contamination with arsenic is a global public health problem, as chronic arsenic exposure can cause serious health damage including cancer. There is also a growing concern regarding the enhancement of arsenic toxicity by lifestyle habit co-exposures, such as smokeless tobacco (SLT) products extensively consumed in Asian countries. Here we studied the in vitro (epi)genomic and phenotypic effects of sodium arsenite (SA), SLT (sadagura) extract, and of SA+SLT combined, to gain new mechanistic insights relevant to oral carcinogenesis. Upon acute exposure, cell viability and genotoxicity were measured in normal oral keratinocyte cultures. Flow cytometry and live-cell imaging analyses were employed to study cell cycle dynamics and apoptosis induction. Temporal transcriptomic analyses by RNAseq addressed the acute exposure effects, while the epigenetic changes following chronic exposure were assessed by DNA methylome analysis.

A dose-dependent decrease in metabolic activity was observed upon individual SA/SLT exposures, an effect exacerbated by co-exposure. Increased γ-H2AX immunofluorescence was observed across exposure conditions and comet assay demonstrated significantly increased DNA tail formation following 24-hr SA+SLT co-exposure compared to the individual treatments. Cell-cycle analyses revealed an increase in the sub-G1 cell population in the SA and SA+SLT exposure groups, suggesting possible cell death, a result manifesting more rapidly upon co-exposure. Annexin V staining and Incucyte analysis combined with caspase inhibition indeed revealed increased apoptotic cell death. Gene expression analysis revealed general upregulation of genes implicated in DNA repair and ROS formation, while we observed upregulation of genes involved in key processes such as apoptosis, ubiquitin-dependent proteolysis, and chromatin organization especially upon SA treatment. Following chronic exposure (4-5 weeks), we observed enhanced DNA hypomethylation, primarily upon the SA and SA+SLT exposures. Associated genes were involved in processes including cell differentiation, apoptosis and ROS response.

The results of our integrated multi-omic and phenotypic analyses offer new mechanistic insights into the action of arsenic and smokeless tobacco, with potential relevance for human populations at risk of oral cancer due to the co-exposure.

Keywords:
Multi-Omics, Mechanisms, Arsenic, Smokeless tobacco.