Overview/abstract

Humans are continuously exposed to mixtures of environmental insults with notable impact on health and disease, including cancer. Understanding the mechanisms underlying the consequences of mixed exposures is a key question in cancer etiology and environmental health. Population and experimental studies show that the carcinogenic potential of DNA damaging agents such as benzo[a]pyrene and solar ultraviolet radiation (UVR) is greatly augmented by arsenic, a substance widely distributed in food and drinking water. Early mechanistic studies established an adverse impact of arsenic on DNA repair capacity and we demonstrated that trivalent arsenite (AsIII) disrupts the zinc finger motifs in key DNA repair proteins, leading to greater DNA damage accumulation. Supplemental zinc restores protein function and reduces the effects of AsIII on UVR-stimulated DNA damage. Using whole genome sequencing (WGS) and advanced computational analysis, our recent work shows that AsIII alone does not generate mutations in human keratinocytes or mouse skin, but dramatically increases mutational burden caused by UVR exposure, with a mutational profile dominated by the characteristic C>T mutations. Our study also identified a feature unique to AsIII and UVR co-exposure that could be detected in human skin tumors. *This foundational work establishes accumulated DNA damage as precursor lesions for mutations that ultimately drive carcinogenesis and highlights the critical need to understand how AsIII amplifies the processes required for conversion of DNA damage to mutations in skin cancer genomes.*

UVR-induced cyclobutane pyrimidine dimers (CPD) are the predominant precursors to mutations in skin cancer, but there is an essential gap in knowledge regarding how arsenic promotes the conversion of CPD DNA damage to C>T mutations and alters the distribution of these mutations in genes that drive skin cancer development. Utilizing innovative integrated state-of-the-art approaches, including DNA damage and mutation mapping in the whole genome at single-base resolution, our new preliminary results show that the dramatic increase in CPDs and C>T mutations by As+UVR versus UVR alone are not randomly distributed across the genome. Instead, C>T mutations are more abundant in gene regulatory regions. Furthermore, our initial study using unbiased bioinformatic approach identified highly mutated genes upon UVR exposure and observed striking effects of AsIII and zinc on gene-specific mutations in skin tumors. Collectively, our findings provide strong scientific rationale for the proposed studies. We will test the overall **hypothesis** that AsIII disruption of nucleotide excision repair (NER) pathway impairs CPD repair and increases CPD retention, resulting in enhanced CPD conversion to C>T mutations in gene-regulatory regions of certain cancer-relevant genes, thus amplifying UVR skin carcinogenesis. We further hypothesize that supplemental zinc will attenuate these AsIII-dependent effects. Three specific aims are proposed:

Aim 1: Determine how AsIII interrupts the NER protein complex and inhibits CPD repair. We hypothesize that AsIII-mediated XPA zinc loss creates a cascade of events in NER that affect the timely recruitments of other proteins, slow down generation of the 30nt repair intermediates, and reduce CPD repair. We will test this hypothesis using confocal microscopy, co-immunoprecipitation with mass spectrometry, and biochemical assays. The findings will clarify the mechanisms by which AsIII disrupts NER to increase CPD accumulation, which is essential for understanding AsIII enhancement of UVR-induced mutations.

Aim 2: Determine the mechanism of AsIII enhancement of CPD conversion to C>T. We will test whether the AsIII-induced delay in CPD repair increases the hydrolytic deamination of cytosines within CPDs as a mechanism leading to base mispairing during DNA replication and promoting C>T mutations. Integration of data from state of the art CPD-seq and WGS will examine the mechanism of AsIII enhancement of CPD conversion to C>T mutations and investigate correlations between CPDs, deaminated CPDs, and C>T mutations within specific genes and functional genomic regions. This will provide the insights into understanding how AsIII modifies conversion of DNA damage to mutations, thus enhancing the development of UVR-induced skin cancer.

Aim 3: Determine gene and pathway impact of AsIII on UVR mutagenesis *in vivo*. We will study the effects of AsIII on CPD retention, deamination, conversion to C>T mutations across the genome and within specific genes using an established in vivo model of AsIII and UVR co-carcinogenesis. Comparative analyses using publicly available human skin cancer genomic datasets will be performed. Additionally, testing the impact of Zn on each of these parameters will provide initial evidence on whether Zn supplementation may represent a viable strategy to offset the profound impact of AsIII on DNA repair and UVR carcinogenesis.

The outcomes from the proposed studies, utilizing both novel CPD-seq and mutation sequencing at the whole genome level with single nucleotide resolution, are expected to have an unprecedented opportunity to provide insight on our mechanistic understanding of arsenic enhancement of UVR mutagenesis and carcinogenesis. Furthermore, establishing evidence that combination exposures are identifiable in the genome has the potential to further our understanding of contributions of mixed environmental factors to human cancer.