TITLE: Defining sites of BPDE-adduct formation and associated breaks across the human genome

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a class of more than 100 chemical compounds derived from a variety of sources, including fossil fuels, engine exhaust, and cigarette smoke. Once in cells, PAHs are oxidized into reactive diol epoxides (PAHDEs) that intercalate into DNA and form covalent linkages that promote mutations and double strand breaks (DSBs) during DNA replication. Although the point mutations produced by PAHs, as well as their targeting to some general sequence characteristics (e.g. G/C rich regions), has been documented, an unbiased genome-wide determination of where PAHs induce DSBs has not been reported. We propose that PAHs generate DSBs through persistent stalling of DNA replication forks, which culminates in their collapse into DSBs. Based on our current findings (Shastri, Tsai et al, *Mol Cell*, submitted), we argue that these DSBs will depend not only on the presence of adducts at the site, but also on the chromatin and sequence context within which stalling occurs.

Our lab has developed techniques to pinpoint sites of replication fork collapse that result from fork stalling (RPA ChIP-Seq and BrITL). In this proposal, we propose to adapt these techniques to: 1) accurately identify the sites of benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) modification of DNA; 2) determine the location of replication fork collapse and DSB generation from benzo[a]pyrene-trans-7,8-dihydrodiol(+/-) (BPDH) exposure; and 3) compare these sites to CNVs and breakpoints in lung cancers that have been linked to cigarette smoking. Sites of BPDE adduct formation will be identified through two complementary methods: BPDE-ChIP and a novel strategy that combines UvrABC cleavage at BPDE linkage sites with cleavage site retrieval by BrITL for next generation sequencing (NGS) and subsequent mapping. Replication fork collapse into DSBs following BPDH exposure will be detected by RPA ChIP-Seq and BrITL in H358 human bronchioalveolar carcinoma (non-small cell lung cancer) cells with and without use of fork collapse sensitizing treatments (ATR inhibition, *Preliminary Studies*). Subsequent studies will use the same assays with normal epithelial cells (HBECs and/or NuLi-1 cells) to assess cellular transformation-dependent differences in site preference, if any. Through these approaches, we will identify where BPDE adducts predominantly occur throughout the genome and, moreover, determine whether some of these sites are more prone to breakage than others. These studies will provide a genomic signature of the effects of benzo[*a*]pyrene (BaP) exposure and determine its relationship to breakpoints in lung cancers attributable to PAHs.