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**Scientific Abstract**

Decreasing prevalence of cigarette smoking in the United States as a result of tobacco reform efforts has coincided with emergence of relatively unregulated cigarette alternatives such as smoking flavored tobacco (shisha) via waterpipe (hookah). Whereas hookah smoke contains a number of carcinogens present in cigarette smoke, epidemiologic associations between hookah tobacco and lung cancer risk have not been firmly established. Furthermore, the effects of hookah smoke in human respiratory epithelial cells have not been investigated in a systematic manner. In published studies, we have utilized in-vitro model systems to identify novel genetic, epigenetic and phenotypic effects of cigarette smoke in normal human respiratory epithelia and lung cancer cells. Molecular alterations induced by CSC in our models have also been observed in primary lung cancer specimens, attesting to the biologic relevance of these surrogate markers of malignancy. We propose to utilize our well-established models to directly examine if hookah smoke mediates cancer-associated alterations in normal respiratory epithelia and lung cancer cells. Briefly, human small airway epithelial cells (SAEC), cdk4/hTERT-immortalized human bronchial epithelial cells (HBEC), and lung cancer cells derived from smokers and never-smokers will be cultured in normal media in the presence or absence of hookah or cigarette smoke condensates under relevant exposure conditions for up to 24 months. Micro-array, quantitative RT-PCR, bisulfite- and pyro-sequencing, chromatin immunoprecipitation, RNA cross-link immunoprecipitation, immunoblot, cell proliferation and nude mouse xenograft experiments will be performed to examine the effects of hookah smoke on DNA methylation, mRNA and microRNA profiles, as well as the histone code relative to those induced by smoke from conventional un-mentholated and mentholated cigarettes. CGH and DNA/RNA sequencing experiments will be performed to evaluate DNA mutations induced by hookah tobacco relative to conventional cigarette smoke. Additional experiments will determine if hookah smoke enhances the malignant phenotype of lung cancer cells; if so, studies will be performed to characterize the mechanisms by which this phenomenon occurs, relative to those recently ascribed to cigarette smoke. These comprehensive integrated studies will leverage expertise from intramural as well as extramural investigators with documented expertise regarding basic and translational research pertaining to tobacco associated airway malignancies, molecular profiling, and biomarker discovery/validation. Results of the proposed studies may provide fundamental insights regarding carcinogenic effects of hookah tobacco, and inform public health officials regarding the need to regulate production, sale and use of this perceived “safe” cigarette alternative.