Tobacco-Induced Transcriptional Dysregulation Associates with Attenuation of Osteogenesis

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Musculoskeletal defects, form the largest group of defects that include head and limb defects, have been associated with tobacco exposure *in utero*. Pregnant women who cannot refrain from smoking are often lured to using harm reduction tobacco products (HRTPs)—which are marketed as safer alternatives. Since proper development of osteoblasts, the bone forming cells, depends on regulatory genes that direct skeletal development, we hypothesized that such genes could be improperly altered when exposed to tobacco products. It is unclear whether transcriptional regulators of osteogenesis are sensitive to HRTPs and whether exposure can result in a skeletal abnormality. We provide here the first evidence that HRTPs are toxic to the developing skeleton. Mice exposed to Camel Blue sidestream (SS) smoke and Camel Snus extracts (STE) *in utero* exhibited defects in bone mineralization and morphological abnormalities, showing decreased cranial and sternum mineralization and maldeveloped ribs. Using human embryonic stem cells (hESCs), Camel Blue and STE decreased osteoblast differentiation. Mechanistically, HRTPs increased levels of reactive oxygen species, concomitant with diminishing superoxide dismutase and catalase activity. FOXO transcription factors, regulators of free-scavenging enzymes, were decreased both at the mRNA and nuclear protein levels due to HRTP exposure. FOXO knockdown cultures phenocopied the reduction of osteoblast production. Globally, RNA-sequencing analysis revealed a divergent mechanisms of osteogenic inhibition between HRTP treated cultures. Camel Blue targeted FOXOs and genes associated with growth receptor signaling (i.e. *EGFR*and *IGF1R*) whereas STE misregulated genes associated with paraxial mesoderm development (*T*, *EOMES*, *CDX1/2*). Combined with ChIP-seq analysis, Gene Ontology terms suggested that the lack of FOXO correlated to defects in embryonic development, skeletal development, osteoblast differentiation, cell fate commitment, and extracellular matrix organization. Overall, our data suggests that HRTPs disrupt embryonic skeletal development by modulation of HRTP-sensitive transcription factors that are regulated early in osteoblast differentiation. Ultimately, this novel data updates the understanding of how smoking affects fetal development.