

## **Quantifying Cigarette Smoke Induced Oxidative DNA Damage of Wild Type & XPC Knockdown Human Bronchial Cells Using Human 8-oxoguanine DNA Glycosylase 1**

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### **Background and Hypothesis:**

Chronic exposure to cigarette smoke (CS) induces DNA lesions and oxidative damage leading to the development of lung diseases such as emphysema and lung cancer. Xeroderma Pigmentosum group C (XPC) is a protein which repairs CS bulky DNA lesions and oxidative DNA damage, including 8-OHdG. We hypothesize that in vitro CS exposure will result in increased 8-OHdG and that XPC knockdown (KD) leads to higher levels of 8-OHdG.

### **Experimental Methods:**

Briefly, immortalized normal human bronchial epithelial cells (Beas2B), were cultured and subsequently exposed to CS extract to induce DNA damage. Cells in agarose were then gently lysed, immersed in an alkaline buffer, incubated with hOGG1 enzyme, and analyzed for DNA damage using single cell electrophoresis (Comet Assay). Slides were stained and viewed by fluorescence microscopy. DNA damage is expressed as tail moment, measured by CometScore. Statistical analysis was performed using 2-way ANOVA (SigmaStat) with p-value < 0.05 indicating statistical significance.

### **Results and Conclusion:**

Both XPC KD and wild-type (WT) Beas2B cells treated with 2.5% CS measured higher levels of DNA damage than air control. The addition of hOGG1 lead to an increase in the tail moment in both AC and CS-exposed cells, indicating an increase in the oxidative DNA lesion, 8-OHdG. Finally, XPC KD exposed to 2.5% CS demonstrated an increase in DNA damage and in 8-OHdG compared to WT (p<0.001). In conclusion, these results further support that CS leads to increased oxidative DNA damage and suggests a role for XPC in regulation and repair of the oxidative DNA lesion, 8-OHdG.