Effects of Prolonged Hexavalent Chromium Exposure on Homologous DNA Recombination in Human Epithelial Cells Grown in Minimal Serum Media

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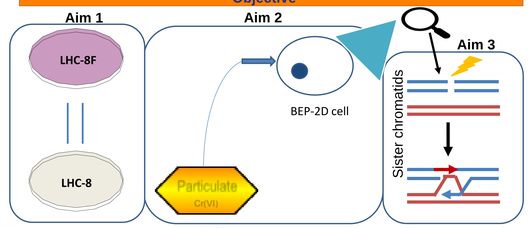
Project Overview

Research Question

Chromium is one of the most heavily used metals in industry. Its hexavalent form Cr(VI), which is usually produced during industrial processing, has been shown to decrease cellular ability to repair double strand DNA breaks leading to chromosomal instability and eventually to cancer, particularly lung carcinomas. Remarkably, pathology data shows that while lung epithelial cells develop into the cancer, the fibroblast of the underlying stromal layer accumulate the Cr, thus suggesting a cell-to-cell interaction is important. Our ultimate goal is to co-culture fibroblasts and epithelial cells together to evaluate their interactions; however, these cells are grown in two very different mediums. Therefore, this project seeks **to characterize the epithelial cell line BEP-2D in a modified low serum media as a baseline for future co-culture models.**

Objective



This study pursues three aims: 1) Demonstrate that no genetic changes occur due to growth in low serum media; 2) Test the concentration of Cr(VI) being imported into the cells; 3) Quantify cellular DNA damage by measuring sister chromatid exchanges.

Take Home Message

BEP-2D cells are genetically stable in in LHC-8F 0.2% CCS media and show a concentration and time dependent response to Cr(VI).

Next Steps

Future work aims at an 3D co-culture model with human fibroblast cells in order to decipher the cell-to-cell interaction between the epithelial and fibroblasts cells in the presence of Cr(VI).

Aim 1: Demonstrate that no genetic changes occurred due to growth in low serum media

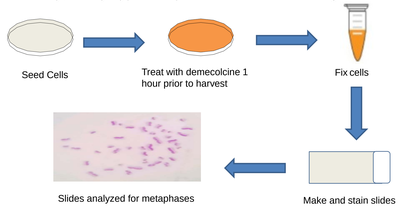
Why we did it:

Epithelial cells are typically grown in serum free media. Fibroblasts are typically grown in media containing 15% serum. Media was developed to support growth of both epithelial cells and fibroblasts. It must be determined if the BEP-2D epithelial cells grown in LHC-8F media with 0.2% cosmic calf serum will differentiate or undergo any genetic changes based solely on change the media.

How we did it:

The initial experiment performed checked for the development of growth foci. BEP-2D cells were seeded into a six well dish. Three of the wells were fed with normal LHC-8 media as a negative control, and the other three wells were fed with LHC-8F media. Cells were grown to confluency and monitored for a loss of cell-to-cell growth inhibition in which the cells would grow overtop of one another. No foci developed in either group.

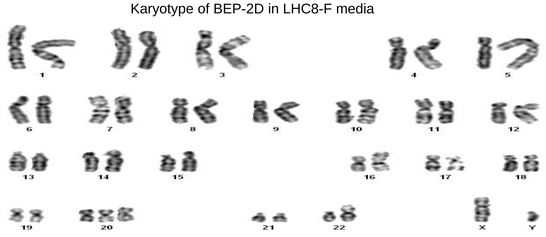
Though the cells showed no apparent transformative changes in the LHC8-F media based on the growth foci assay, a karyotype was performed to monitor for genetic changes.



Cells were seeded into tissue culture dishes and allowed to rest for 3 days. They were then treated with demecolcine 1 hour prior to harvest in order to arrest cells in metaphase. After slides were made, they were stained for banding and a karyotype was performed.

What we found:

Karyotype of BEP-2D in LHC8-F media



What does it mean?

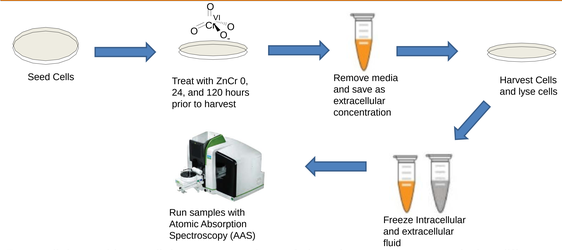
The growth of the BEP-2D in the low serum LHC-8F did not alter the genetic make-up of the cells when compared to BEP-2D cells grown in normal LHC-8 media.

Aim 2: Test the concentration of Cr(VI) being imported into the cells

Why we did it:

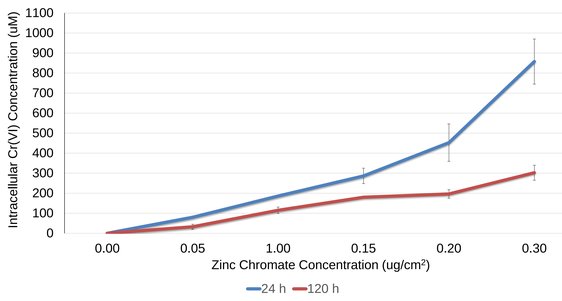
In order to interpret any effects of Cr(VI) on BEP-2D cells, the level of intracellular chromium after exposure needs to be measured. The metal ion uptake assay measures the amount of chromium inside the cells after treatment with different concentrations of Cr(VI).

How we did it:



Extracellular and intracellular concentrations of chromium were measured after different treatment concentrations and exposure times.

What we found:



BEP-2D cells show a concentration dependent increase in the amount of intracellular chromium; however, less chromium was present after 120 hours compared to 24 hours.

What does it mean?

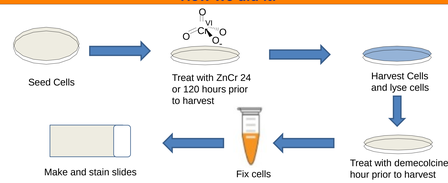
The concentration dependent increase was expected as increased chromium influx would follow increased chromium availability. This means that at higher exposure concentrations, there is a greater risk of chromosomal instability and, therefore, cancer. The time dependent decrease presents surprisingly and could be due to an efflux mechanism present in the BEP-2D cells, but follow up studies will be required.

Aim 3: Quantify cellular DNA damage by measuring sister chromatid exchange rate

Why we did it:

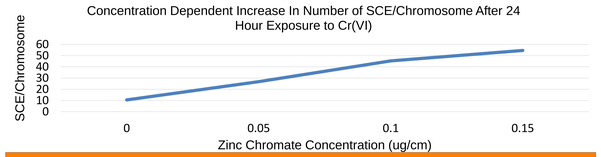
Sister chromatid exchanges occur through homologous DNA recombination repair after double stranded DNA breaks; therefore, we could gauge the relative amount of DNA damage caused by the Cr(VI) by the evidence of DNA repair in the form of sister chromatid exchanges.

How we did it:



Cells were seeded in tissue culture dishes where they were treated with varying concentrations of ZC at either 24 hours or 120 hours. The cells were treated with BrdU roughly 2 cell cycles prior to harvest and demecolcine 1 hour prior to harvest. Cells were then dropped onto slides and stained. Analysis involved counting the SCE events per chromosome in 50 metaphases at each ZC concentration and time point.

What we found:



What does it mean?

Analysis to this point shows a concentration dependent increase in the number of sister chromatid exchanges in the BEP-2D cells. Based on previous experiments in other cell lines, we expect to see both a concentration and time dependent increase.

Further Reading

Qin Q, Xie H, Wise SS, et al. Homologous Recombination Repair Signaling in Chemical Carcinogenesis: Prolonged Particulate Hexavalent Chromium Exposure Suppresses the Rad51 Response in Human Lung Cells. *Toxicological Sciences*. 2014;142(1):117-125.

Hosoya N, Miyagawa K. Targeting DNA damage response in cancer therapy. *Cancer Science*. 2014;105(4):370-388.

Browning CL, Wise JP Sr: Prolonged exposure to particulate chromate inhibits RAD51 nuclear import mediator proteins. *Toxicol Appl Pharmacol.* 2017; **331**: 101–7.

Browning CL, Qin Q, Kelly DF, *et al.*: Prolonged Particulate Hexavalent Chromium Exposure Suppresses Homologous Recombination Repair in Human Lung Cells. *Toxicol Sci.* 2016; 153(1): 70–8.

Acknowledgements

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