The Effects of Whole Life, Low Dose Cadmium Exposure on Mouse Lung Histology and DNA Damage

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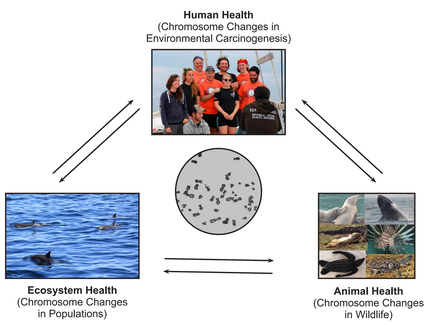
A One Environmental Health Approach

A



B

A) We pursue a One Environmental Health approach, focusing on genomic instability.

B) We mimic occupational and environmental heavy metal exposure, which requires long term administration of low doses. We use chemical carcinogenesis tools and 'Omics' technologies combined with the One Environmental Health approach (i.e. using wildlife and ecosystem health to inform about human health) to gain insight into the function, persistence, and cellular heritability of metal-induced genomic instability and changes in DNA damage repair and their roles in lung cancer. We translate discoveries in our cell culture models to responses in animal models. The integration of our model systems, from cells and rats to human and whales is important to answering our research questions about the molecular mechanisms for metal-induced lung cancer.

Further Reading

Liang Y, Young JL, Kong M, Tong Y, Qian Y, Freedman JH, Cai L. Gender Differences in Cardiac Remodeling Induced by a High-Fat Diet and Lifelong, Low-Dose Cadmium Exposure. Chem Res Toxicol. 2019 Jun 17;32(6):1070-1081. doi: 10.1021/acs.chemrestox.8b00386

Zhang Y, Young JL, Cai L, Tong YG, Miao L, Freedman JH. Chronic exposure to arsenic and high fat diet induces sex-dependent pathogenic effects on the kidney. Chem Biol Interact. 2019 Jun 22;310:108719. doi: 10.1016/j.cbi.2019.06.032.

Chen C, Xun P, Nishijo M, He K. Cadmium exposure and risk of lung cancer: a meta-analysis of cohort and case-control studies among general and occupational populations. J Expo Sci Environ Epidemiol. 2016 Sep;26(5):437-44. doi: 10.1038/jes.2016.6. Epub 2016 Mar 9.

Project Overview

Research Question

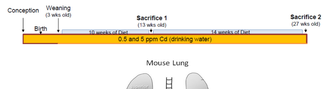
Lung cancer is the leading cause of cancer deaths amongst the population. Cigarette smoking is considered the major cause of lung cancer, but there are a number of other factors that account for this disease, such as environmental exposures through metals. Cadmium is a heavy metal and environmental contaminant that is a well-known human health concern and associated with a number of health effects, including cancer. It is naturally occurring and widely used in industrial productions. For non-smokers, the main source of cadmium exposure is through diet. Other heavy metals, including hexavalent chromium and arsenic, are well known to induce lung cancer. Recently, assessment data support an independent effect for cadmium in risk of lung cancer mortality in cadmium and arsenic occupational workers. One of the major events leading to heavy metal carcinogenesis is DNA damage. Therefore, this project asks: **Does cadmium induce DNA damage within the lungs of whole life, low dose exposed mice?**

Overall Study Design

A

In Utero and Pre-Weaning Exposure

Post-Weaning Exposure



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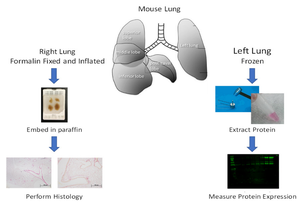


A) Schematic for whole life, low dose cadmium exposure in mice.
B) Lungs from 27 week old mice were collected and the right lung was formalin fixed, paraffin embedded for histology studies and the left lung was frozen for protein extraction.

Take Home Message

Cadmium may induce histological changes that could lead to a carcinogenic outcome. More studies are needed to verify the effect of cadmium on DNA damage.

Next Steps

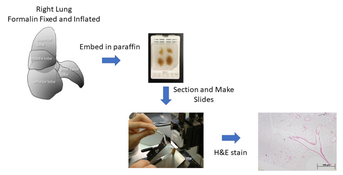
Future work aims at continuing the analysis of expression levels of various proteins involved in the repair of DNA damage. Any proteins showing changes in expression will be analyzed for changes in RNA levels. Changes in chromosome instability will also be assessed. Results will lead to the first reports of the impact of cadium on DNA damage and repair in the lung.

Aim 1: Characterize Cadmium Induced Changes in Cellular Structure and Morphological Aberrations

Why we did it:

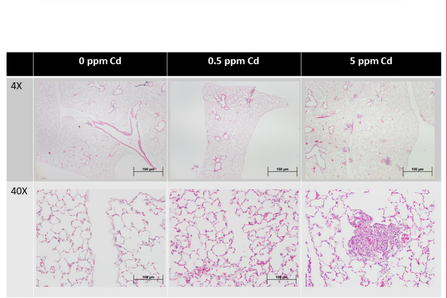
As an intital characterization of the overall effects of cadium on the lung, we performed histological analysis of formalin fixed parrafin embedded lungs. This analysis will reveal any histological changes that could lead to carcenogenic outcomes.

How we did it:



The right lung of 27 week old mice whole-life exposed to cadmium was collected, fixed and inflated in formalin. After embedding the lung in paraffin, 5 um sections were cut and mounted on slides. Slides were then H&E stained. Qualitative analysis was then performed to look for any abberations in cell morphology and growth.

What we found:



Sections from mice lungs treated with 0, 0.5, or 5 ppm cadmium were H&E stained to look at cellular morphology. No changes in cell proliferation or morphology were seen at 0.5 ppm cadmium. At 5 ppm cadmium, changes in cell histology were seen. These changes were seen throughout the lung sections and showed areas consistent with the development of adenomas.

What does it mean?

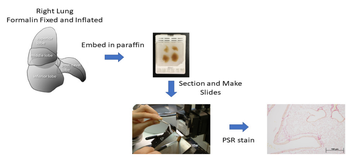
Structural changes were seen in the 5ppm high dose cadmium exposure group compared to that of the control group. Alterations include several adenomas seen in multiple lobes of the lung. These data suggest low dose chronic cadmium exposure may induce structural changes in lung tissue consitent with a carcenogenic outcome.

Aim 2: Effect of Cadmium Induced Tissue Remodeling and Repair

Why we did it:

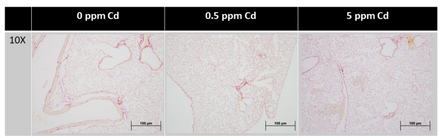
As an intital characterization of the overall effects of cadium on the lung, we performed fibrosis analysis of formalin fixed parrafin embedded lungs. This analysis will reveal any changes in the amount of collagen present, indicating progression towards inflammation or other disease states.

How we did it:



The right lung of 27 week old mice whole-life exposed to cadmium was collected, fixed and inflated in formalin. After embedding the lung in paraffin, 5 um sections were cut and mounted on slides. Slides were then PSR stained. Qualitative analysis was then performed to look for any increase in collagen.

What we found:



Sections from mice lungs treated with 0, 0.5, or 5 ppm cadmium were PSR stained to look increases in the presence of collagen. No changes in the levels of collagen were qualitatively seen at 0.5 ppm or 5 ppm cadmium treated mice.

What does it mean?

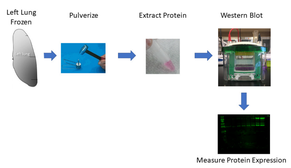
PSR staining analyses between the control groups and the cadmium exposed groups showed no significant difference in the amount of collagen present in the lung tissue. These data indicate that cadmium exposure may not induce fibrosis or inflammation to cause tissue remodeling.

Aim 3: Measure Cadmium Induced DNA Damage

Why we did it:

One of the major events leading to heavy metal carcinogenesis is DNA damage. To determine if cadmium exposure leads to DNA Damage, protein levels of DNA damage marker H2AX were measured in lung tissue from mice exposed to cadmium.

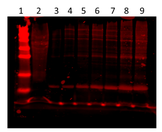
How we did it:



The left lung of 27 week old mice whole-life exposed to cadmium was collected and frozen. The frozen lung tissue was then pulverized to a homogenous powder and protein extracted. The extracted protein lysate was then run on an SDS-PAGE gel for protein seperation. Western blot analysis was then performed for the DNA damage marker protein H2AX.

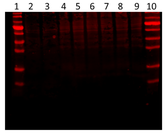
What we found:

A Testing H2AX antibody reactivity in cultured mouse cells



1. Ladder
2. 5 ug +2C
3. 5 ug
4. 10 ug
5. 20 ug
6. 40 ug
7. 50 ug
8. 80 ug
9. 100 ug

B H2AX Western Blot from Cadmium Treated Mice



1. Ladder
2. Male 0 ppm Cd
3. Female 0 ppm Cd
4. Male 0.5 ppm Cd
5. Female 0.5 ppm Cd
6. Female 0.5 ppm Cd
7. Male 5 ppm Cd
8. Female 5 ppm Cd
9. Female 5 ppm Cd
10. Ladder

A) Testing increasing concentrations of cell lysate from cultured mouse cells shows that the H2AX antibody can react to the mouse antigen and signal increases as protein concentration increases.
B) Western blot of pulverized tissue extract from lungs of cadmium exposed mice show no reactivity for the H2AX antibody.

What does it mean?

H2AX levels in mouse tissue were not able to be measured. Further analysis and optimization are needed in the western blot to determine the effects of cadmium on DNA damage.

Acknowledgements

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