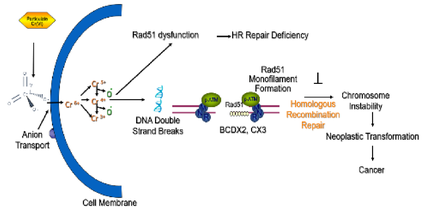
Particulate Hexavalent Chromium Exposure Induces Persistent and Heritable Loss of RAD51D

Catherine H. Doyle, Aggie R. Williams, Cynthia L. Browning, Sandra S. Wise and John Pierce Wise, Sr.

Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, United States

Overview

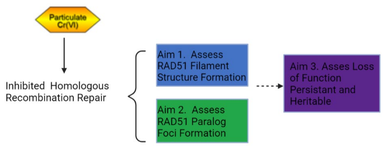


Hexavalent chromium is a toxic and carcinogenic environmental contaminant which has been associated with genomic instability and DNA double strand breaks. DNA double strand breaks are repaired by homologous recombination repair (HR). The defining step of HR is the formation of nucleoprotein filaments which assists in the location of the homologous sequence and template invasion. RAD51 and its paralog proteins are integral in this process, though previous research shows Cr(VI) inhibits their function. Without HR repair DNA double strand breaks can occur causing genetic instability and neoplastic transformation in some cases.

Research Question

Previous research has shown after an acute exposure (24 hours) to hexavalent chromium the regular HR repair pathway is triggered, but after a prolonged exposure (>72 hours) there is a significant decrease in HR repair. RAD51 forms nucleoprotein filaments with the help of its paralog proteins. Since their formation is critical to proper HR repair, RAD51 has been a target of research concerning DNA double strand break repair due to Cr(VI) exposure. To further investigate the relationships between the RAD51 paralogs and HR repair, we chose our main research questions: **Does Cr(VI) inhibit the function of RAD51 and paralog proteins? If so, are these impairments persistent and heritable across generations?**

Overall Study Design



In order to answer these questions, we looked at how RAD51 and its paralog proteins were affected by hexavalent chromium exposure in three aims. We looked at these aims using cell cultures of WTHBF-6 human lung fibroblast cells and varying concentrations and exposure times of zinc chromate:

1. Assess RAD51 Filament Structure Formation after Cr(VI) Exposure
2. Assess RAD51 Paralog Foci Formation after Cr(VI) Exposure
3. Assess Whether RAD51D Loss of Function is Persistent and Heritable using three generations of clonal cell lines exposed to Cr(VI) for three sequential 24-hour periods between each generation being seeded

Take Home Message

Particulate hexavalent chromium causes decreases in HR by impairing the structure and function of RAD51 and its paralog proteins. These impairments can be persistent and heritable after just 24 hours of exposure, as shown in RAD51D.

Aim 1: Assess RAD51 Filament Structure Formation after Cr(VI) Exposure

Why we did it

Previous research showed HR repair is inhibited by particulate Cr(VI) exposure. In order to understand how Cr(VI) inhibits HR repair, we looked at how acute and prolonged exposure affected RAD51 filament and foci formation.

How we did it

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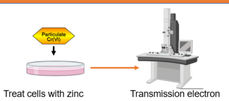

B In 100 mm plates, cells were seeded and treated with zinc chromate for 24 or 120 hours. After the treatment was complete, the cells were washed twice with PBS and the cell pellets were flash frozen in liquid nitrogen. Transmission electron microscopy with affinity capture were utilized to visualize RAD51 filaments in a 1 grid square (37 μm^2). The nucleoprotein filaments were categorized as 'simple' or 'complex' structures.

What we found

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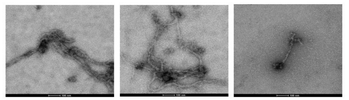

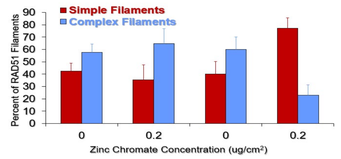

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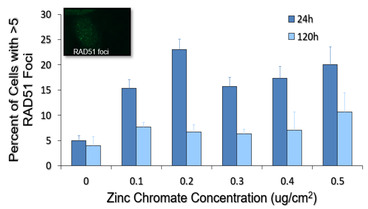

A. This figure shows the number of complex RAD51 filament structures decreases while the number of simple structures increases after prolonged hexavalent chromium exposure.
B. This figure shows RAD51 foci formation is inhibited after prolonged hexavalent chromium exposure.

What does it mean

Our data suggest RAD51 nucleoprotein filament formation is inhibited by prolonged particulate hexavalent chromium exposure, which prevents cells from performing HR.

Aim 2: Assess RAD51 Paralog Foci Formation after Cr(VI) Exposure

Why we did it

Since we saw structural impairments in nucleoprotein filament formation and a decrease in RAD51 foci, we chose to investigate the RAD51 paralog proteins. RAD51 loading and function is assisted by two complexes comprised of paralog proteins. We wanted to assess whether these paralogs were inhibited by hexavalent chromium. We chose one paralog from each complex (RAD51D and XRCC3) and RAD41C, which is in both complexes.

How we did it

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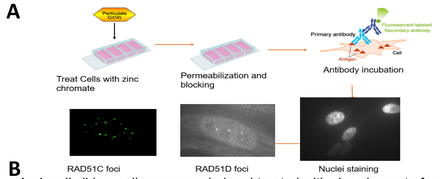

B In 4-well slides, cells were seeded and treated with zinc chromate for 24, 72, 120 hours. The cells were then fixed, permeabilized, and blocked with blocking buffer. PBS rinses were used between each step to remove any debris from the cells. After blocking, the primary antibody was incubated for an hour. After being washed three times, the secondary antibody was added to the cells and allowed to incubate for an hour. The slides were left to dry in the dark. DAPI nuclei stain was added and allowed to dry. A confocal microscope was then utilized to visualize the cells and the number of foci per cell was scored for 100 cells per well.

What we found

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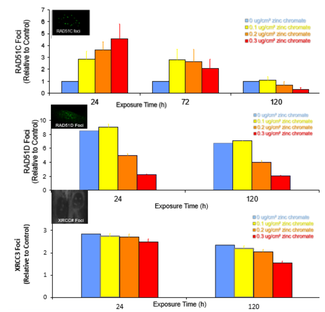

B This figure shows acute hexavalent chromium exposure increased Rad51C and XRCC3 foci formation but prolonged exposure inhibited foci formation. Additionally, both acute and prolonged exposure reduced RAD51D foci formation.

What does it mean

Our data indicate the RAD51C and XRCC3 paralogs are only inhibited after prolonged hexavalent chromium exposure, while the RAD51D paralog is affected by both prolonged and acute exposure. This outcome suggests RAD51D and the BCDX2 complexes are targeted by Cr(VI) causing diminished HR repair.

Aim 3: Assess Whether RAD51D Loss of Function is Persistent and Heritable

Why we did it

We chose to focus on RAD51D because our data show both acute and prolonged Cr(VI) exposure affect its foci formation unlike XRCC3 and RAD51C which were only affected after prolonged exposure. In order to see if this effect was permanent, we chose to look at the inhibition persistence in clonal cell lines. This allowed us to look at if Cr(VI) foci inhibition is persistent and heritable to future generations.

How we did it

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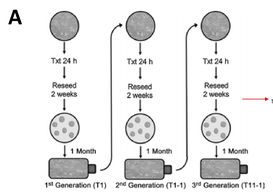

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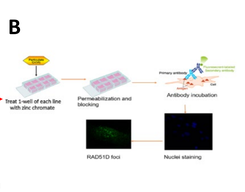
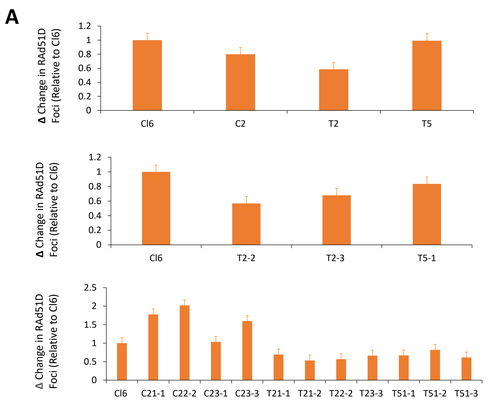


A. Three generations of clonal cell lines were created using single seeding colony forming densities. B. In 8-well slides, cells were seeded with each cell line in two wells. One well per line was treated with zinc chromate for 24 hours. The cells were then fixed, permeabilized, and blocked with blocking buffer. PBS rinses were used between each step to remove any debris from the cells. After blocking, the primary antibody was incubated for an hour. After being washed three times, the secondary antibody was added to the cells and allowed to incubate for an hour. The slides were left to dry in the dark. DAPI nuclei stain was added and allowed to dry. A comet microscope was then utilized to visualize the cells and the number of foci per cell was scored for 100 cells per well.

What we found

A



B

Decreases in RAD51D foci formation are persistent after the removal of hexavalent chromium across multiple cell lines. Additionally, this decrease is heritable to clonal generations.

What does it mean

Our data indicate RAD51D foci formation shows increased impairment with each successive generation. This suggests this impairment of RAD51D is both persistent and heritable after acute Cr(VI) exposure.

Acknowledgements

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Further Reading

Browning, C.L., Qin, Q., Kelly, D.F., Prakash, R., Jasin, M., and Wise, Sr., J.P. Prolonged Particulate Hexavalent Chromium Exposure Suppresses Homologous Recombination Repair in Human Lung Cells. "Toxicological Sciences." 2016,123(1):70-78.

Qin, Q., Xie, H., Wise, S.S., Browning, C.L., Thompson, K.N., Holmes, A.L., and Wise, Sr., J.P. 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.