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Homologous Recombination Repair Protects Against Particulate Chromate-induced Chromosome Instability in Chinese Hamster Cells

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Abstract

Particulate hexavalent chromium [Cr(VI)] compounds are well-established human carcinogens. Cr (VI)-induced tumors are characterized by chromosomal instability (CIN); however, the mechanisms of this effect are unknown. We investigated the hypothesis that homologous recombination (HR) repair of DNA double strand breaks protect cells from Cr(VI)-induced CIN by focusing on the XRCC3 and RAD51C genes, which play an important role in cellular resistance to DNA double strand breaks. We used Chinese hamster cells defective in each HR gene (irs3 for RAD51C and irs1SF for XRCC3) and compared with their wildtype parental and cDNA-complemented controls. We found that the intracellular Cr ion levels varied among the cell lines after particulate chromate treatment. Importantly, accounting for differences in Cr ion levels, we discovered that XRCC3 and RAD51C cells treated with lead chromate had increased cytotoxicity and chromosomal aberrations, relative to wild-type and cDNA-complemented cells. We also observed the emergence of high levels of chromatid exchanges in the two mutant cell lines. For example, 1 ug/cm² lead chromate induced 20 and 32 exchanges in XRCC3- and RAD51C-deficient cells, respectively, whereas no exchanges were detected in the wildtype and cDNA-complemented cells. These observations suggest that HR protects cells from Cr(VI)-induced CIN, consistent with the ability of particulate Cr(VI) to induce double strand breaks.

Keywords

chromate; particulate; XRCC3; RAD51C; chromosome instability; double strand break; homologous recombination

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1. Introduction

Particulate hexavalent chromium Cr(VI) compounds are well-established human lung carcinogens. Chromosome instability (CIN) is a hallmark of lung cancer and recent data extend these observations to include Cr(VI)-induced tumors [1–4]. Particulate Cr(VI) compounds are considered to be the more potent carcinogenic form of Cr(VI), associated with a greater risk of lung cancer in exposed workers, and an increased incidence of tumors in experimental animals and neoplastic transformation of cultured cells [5–7].

Particulate Cr(VI) has been shown to cause CIN manifested as aneuploidy and chromosome aberrations [8]. The physicochemical mechanism indicates that the Cr(VI) particles partially dissolve outside the cells, releasing the chromate anion and a cation [9]. The chromate anion readily enters the cell via facilitated diffusion and is immediately reduced to trivalent chromium. The products of this reduction then induce DNA damage, growth arrest and cytotoxicity, while the cation (e.g. lead) has no apparent effect [9–13]. Soluble and particulate Cr(VI) compounds (lead chromate, potassium dichromate, calcium chromate, and chromium oxide, sodium chromate) induce cytotoxicity, mutation to 6-thioguanine resistance, and anchorage independence in cultured diploid human fibroblasts at μM concentrations, but Cr(III) compounds only cause cytotoxicity and weak mutagenicity at 100–1,000 fold higher concentrations (14–15). However, while the physicochemical mechanism is understood, the genetic mechanisms that protect cells from particulate Cr(VI)-induced CIN, manifested as structural chromosomal aberrations, are not characterized.

Particulate Cr(VI) has been shown to cause DNA single-strand and double-strand breaks, DNA adducts, and DNA-protein crosslinks. Several studies utilizing vitamin pretreatments have shown that adducts and crosslinks do not correlate with chromosomal aberrations [16–19]. DNA single strand breaks are very short-lived after particulate Cr(VI) exposure and thus do not seem like likely candidates for inducing chromosome damage [13]. By contrast, particulate Cr(VI)-induced DNA double strand breaks are more persistent and are known to cause chromosomal aberrations. Thus, we hypothesize that DNA double strand break repair mechanisms play an important functional role in protecting cells from particulate Cr(VI)-induced CIN. Importantly, we have found previously that the DNA double strand break repair pathway of non-homologous end joining (NHEJ), a key mechanism used by the cell to repair DNA double strand breaks, plays no role in protecting the cell from particulate Cr(VI) induced chromosomal damage [20].

Homologous recombination (HR) repair utilizes the presence of the sister chromatid as a template to repair DNA double strand breaks. HR has 3 major steps: Presynapsis (strand stabilization), synapsis (strand invasion and branch migration) and postsynapsis (Holliday junction formation and resolution). Briefly, during HR repair, DNA double strand breaks, particularly those arising during DNA replication, are converted to 3' single-stranded DNA tails, which are bound by RPA. Rad52, along with the five Rad51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3) interact with RPA and promote binding of RAD51 to the single-stranded DNA and stabilization of the nucleoprotein filament. Subsequently, the RAD51-bound single-stranded DNA invades a homologous molecule in a reaction stimulated by RAD54. This invasion leads to Holliday junction formation which is thought to be resolved by a complex containing XRCC3 and RAD51C [21–22].

A recent study has reported increased nuclear RAD51 foci (indicative of formation of RAD51 nucleoprotein filaments and the induction of HR repair after exposure to soluble Cr(VI) [23]. The study also found that cells deficient in XRCC3 or BRCA2 (another protein necessary for RAD51 filament formation) were hypersensitive to the cytotoxic effect of Cr(VI). However, the relative amount of chromium uptake in each of these cell lines was not considered and thus

it is unclear if these data reflect true sensitivity or perhaps a simple difference in chromium uptake. In addition, studies of chromosome aberrations, which reflect the events associated with the carcinogenic effects of Cr(VI) were not performed in these cell lines.

XRCC3 is thought to participate in the resolvase step of HR repair and RAD51C is thought to participate in both the resolvase step and in presynapsis [24–25]. It is unknown if either of these genes are involved in preventing Cr(VI)-induced CIN. Thus, the purpose of our study was to examine the importance of HR repair in preventing particulate chromate-induced CIN by examining the impact of XRCC3 or RAD51C deficiency on the amount and spectrum of particulate Cr(VI)-induced chromosome damage.

2. Materials and Methods

2.1 Chemical and Reagents

Lead chromate, colcemid and potassium chloride (KCl) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dodecyl sulfate (SDS) was purchased from American Bioanalytical (Natick, MA). Giemsa stain was purchased from Biomedical Specialties (Santa Monica, CA). Crystal violet, methanol and acetone were purchased from J.T. Baker (Phillipsburg, NJ). Gurr's buffer, Trypsin-EDTA, sodium pyruvate, penicillin/streptomycin, and L-glutamine were purchased from Invitrogen Corp. (Carlsbad, CA). Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture were purchased from Mediatech Inc. (Herndon, VA). Cosmic calf serum was purchased from Hyclone (Logan, UT). Tissue culture dishes, flasks and plasticware were purchased from Corning Inc. (Acton, MA).

2.2 Cells and Cell Culture

The cell lines used in this study are listed in Table 1. We used the well-characterized XRCC3-deficient Chinese hamster ovary (CHO) cell line model system of irs1SF [26], and the XRCC3 cDNA complemented 1SFwt8 [27] cell lines to examine the role of XRCC3. The effect of Cr(VI) on the CIN of the wildtype parental AA8 CHO cells, from which the irs1SF cells were derived, has been characterized previously [28].

To study the RAD51C gene, we used the model Chinese hamster lung (CHL) fibroblast cell lines: V79, irs3 and irs3#6. The irs3 cell line, derived from the parental V79 cells, is deficient in RAD51C gene, and are used with their cDNA-complemented cell line irs3#6 [29–30]. For further information, we have previously published the details and validation of these cells elsewhere [27–30].

All cells were routinely cultured in DMEM/F-12 supplemented with 10% Cosmic calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.1 mM sodium pyruvate. Cells were maintained at 37 °C as adherent subconfluent monolayers. This was done by subculturing at least twice weekly using 0.25% trypsin/1 mM EDTA solution.

2.3 Chemical Preparation

Lead chromate (CAS #7758-97-6; ACS reagent minimum 98% purity) was used as a model for particulate chromium salt. It was suspended in acetone and administered to cells as previously described [8,31].

2.4 Cytotoxicity Assay

A clonogenic assay was used to measure cytotoxicity. Briefly, this was done by treating logarithmically growing cells with lead chromate for 24 h. Cells were then reseeded to a colony forming density and grown for 6 to 7 days to allow colony formation. Each dish was fixed with

95% EtOH, stained with crystal violet and colonies were counted [30–31]. Each treatment group contained four dishes with at least three independent experiments per cell line.

2.5 Chromosome Preparations

Determination of chromosomal damage was measured as previously described [32–33]. Briefly, logarithmically growing cells were treated with lead chromate for 24 h. Cells were then harvested, resuspended in hypotonic solution and fixed with Carnoy's fixative, dropped onto slides, stained with Giemsa, and cover slips were added. Background damage levels were subtracted from each data point as previously described [32–33]. Chromosomes were analyzed using our published methods [8]. Gaps and breaks were pooled as 'lesions' as previously described [8]. This was done because breaks can only be unequivocally distinguished from gaps if the distal acentric fragment is displaced. Thus pooling aberrations avoids artificial discrepancies between scorers due to different perceptions of the width of a gap relative to the width of its chromatid. Accordingly, chromatid deletions and achromatic lesions were pooled as chromatid lesions. One hundred metaphases were scored for each experiment and all experiments were repeated at least three times for each cell line.

Additionally, a mitotic index was performed without addition of colchicine. There was no difference in the frequency of mitotic cells between the wild-type, gene deficient or gene complemented cells after lead chromate exposure (data not shown).

2.6 Intracellular Chromium Ion Measurement

Analysis of the intracellular chromium ion levels was completed using an inductively coupled plasma- optical emission spectrometer (ICP-OES) as previously described [33]. Briefly, logarithmically growing cells were treated with lead chromate for 24 h. Cells were rinsed with PBS, followed by the addition of hypotonic and then 2% SDS. This solution was then sheared through a needle seven times and filtered through a 0.2 μ m filter. Each experiment was repeated at least three times for each cell line.

2.7 Data analysis

The relationships between dose and cell type and each of the dependent variables were assessed using ANOVA and linear regression models. Indicator variables for dose levels and cell types, as well as product variables for the joint effects of dose level and cell type, were included as independent variables in the models. Differences were evaluated using t-tests and 95% confidence intervals. The relationship between dose and intracellular concentration was quantified using a separate regression analysis for each cell type. The resulting values and variance-covariance matrix were then used to estimate the differences among cell types at concentrations of 100, 500, 1000, and (for cytotoxicity only) 5000 [33]. All analyses were conducted using the SAS software package.

3. Results

3.1 XRCC3-deficient cells have lower intracellular ion uptake of chromium (Cr), but RAD51C-deficient cells have greater uptake compared to parental cells

As a first step we measured intracellular Cr ion levels in all six cell lines used in this study to detect any differences in the amount of Cr entering the cells after lead chromate exposure. Cr ion levels were significantly lower in the *irs1SF* (XRCC3-deficient) cells than AA8 (wildtype) and 1SFwt8 (XRCC3-cDNA complemented cells) (Figure 1A). For example, after a 24 h exposure to 1 μ g/cm² lead chromate, intracellular Cr ion levels were 230 μ M in *irs1SF* cells compared to 817 μ M in AA8 and 700 μ M in 1SFwt8 cells.

By contrast, Cr ion levels were higher in *irs3* (RAD51C-deficient) cells relative to their parental (V79) or RAD51C-cDNA complemented (*irs3#6*) cells (Figure 1B). For example, after a 24 h exposure to 1 $\mu\text{g}/\text{cm}^2$ lead chromate, intracellular Cr ion levels were 1,431 μM in *irs3* cells compared to 664 μM in V79 and 528 μM in *irs3#6* cells. Accordingly, because of the differential Cr uptake among the cell lines we considered administered concentrations and intracellular ion levels for each experimental endpoint.

3.2 Lead chromate induced higher cytotoxicity in XRCC3- and RAD51C-deficient cells versus parental cells

Lead chromate induced a clear concentration-dependent decrease in the relative survival of the *irs1SF* XRCC3-defective cells and their controls, (Figure 2A). Correcting for intracellular Cr levels show that the XRCC3-deficient cells are significantly more sensitive to the cytotoxic effects of lead chromate (Figure 2B). For example at an intracellular Cr level of 100 μM , the XRCC3-deficient cells show about 70% relative survival compared to 85% survival in the wildtype (V79) (p value: <0.0001). The XRCC3 cDNA-complemented cells show a decrease in sensitivity to the cytotoxic effects of chromium relative to the deficient cells.

RAD51C-deficient cells also showed increased sensitivity to cytotoxicity by exposure to Cr. Lead chromate caused a concentration-dependent decrease in the relative survival of all three CHL cell lines tested (V79, *irs3* and *irs3#6*; Figure 2C), but correcting for intracellular Cr levels show that the RAD51C-deficient cells are more sensitive to the cytotoxic effects of lead chromate (Figure 2D). For example at an intracellular Cr level of 500 μM the RAD51C-deficient cells show about 63% relative survival compared to 83% survival in the parent (V79) (p value: <0.0001). RAD51C-complemented cells were restored to wild-type levels of sensitivity (p value: <0.1444).

3.3 Lead chromate increased frequency of metaphases with chromosome damage in XRCC3- and RAD51C-deficient cells

Lead chromate induced a concentration-dependent increase in the percentage of metaphases with at least 1 broken chromosome in all three XRCC3-related cell lines (Figure 3A). For example, 1 $\mu\text{g}/\text{cm}^2$ lead chromate induced chromosomal aberrations in 20% of XRCC3-deficient metaphases compared to 8% in parental (p value: <0.0345). XRCC3-complemented cells were restored to wild-type (p value: <0.3961). Correcting for differences in intracellular Cr levels showed an even greater effect (Figure 3B). For example, at an intracellular Cr level of 100 μM , XRCC3-deficient cells had 17% metaphases with damage compared to 3% in the parental (p value: <0.0019). XRCC3 cDNA-complemented cells were restored to wild-type levels of chromosome damage (p value: <0.5839).

Lead chromate also induced a concentration-dependent increase in the percent of damaged metaphases in all three RAD51C-related cell lines (Figure 3C). For example, 1 $\mu\text{g}/\text{cm}^2$ lead chromate induced chromosomal aberrations in 45% of RAD51C-deficient metaphases compared to 8% in parental (p value: <0.0001). RAD51C-complemented cells showed 4% of metaphases with aberrations, similar to the parental cells (p value: <0.3833). Correcting for differences in intracellular Cr levels showed the same trend (Figure 3D). For example, at an intracellular Cr level of 500 μM , RAD51C-deficient cells had 20% metaphases with damage compared to 5% in the parental (p value: <0.0001). RAD51C-complemented cells were restored to wildtype (p value: <0.6171).

Next, we assessed total chromosomal damage, which reflects the total number of aberrations in 100 metaphases and provides a measure of cells with more than 1 chromosome aberration. As expected, we found that lead chromate induced a concentration-dependent increase in total amount of chromosome damage in the XRCC3-deficient *irs1SF* cells and their control cell

lines (Figure 4A). For example, 1 $\mu\text{g}/\text{cm}^2$ lead chromate induced 28 chromosomal aberrations in 100 XRCC3-deficient metaphases compared to 8 in parental (p value: <0.0603). XRCC3-complemented cells had 6 chromosomal aberrations which were similar to the wild-type (p value: <0.5902). Due to the reduced uptake of Cr by the *irs1SF* cells, correcting for differences in intracellular Cr levels showed an even greater effect (Figure 4B). At an intracellular Cr level of 100 μM , XRCC3-deficient cells had 20 aberrations in 100 metaphases with damage compared to 3 in the parental (p value: <0.0213). XRCC3 gene-complemented cells were restored to wild-type levels of total chromosomal aberrations per Cr dose (p value: <0.7773).

Lead chromate also induced a concentration-dependent increase in the total chromosome damage in RAD51C-deficient *irs3* cell line and its controls (Figure 4C). For example, 1 $\mu\text{g}/\text{cm}^2$ lead chromate induced 68 chromosomal aberrations in 100 RAD51C-deficient metaphases compared to 10 in parental (p value: <0.0001). RAD51C-complemented cells showed 5 aberrations per 100 metaphase spreads, similar to the wildtype cells (p value: <0.6033). Correcting for differences in intracellular Cr levels showed the same trend (Figure 4D). For example, at an intracellular Cr level of 500 μM , RAD51C-deficient cells had 38 aberrations in 100 metaphases with damaged compared to only 5 in the parental cells (p value: <0.0001). RAD51C-complemented cells were restored to wildtype levels of aberrations (p value: <0.8065).

3.4 Lead chromate induces chromatid exchanges in XRCC3- and RAD51C-deficient cells

Finally, we considered effects of XRCC3 and RAD51C deficiencies on the spectrum of chromatid-type damage. Lead chromate caused an overall increase in chromatid lesions in both Rad51C-paralog-deficient cell lines studied compared to the corresponding wildtype and gene-complemented cells (Figure 5). However, even more remarkable was that lead chromate induced a high level of chromatid exchanges in XRCC3- and RAD51C-deficient cells. For example, 1 $\mu\text{g}/\text{cm}^2$ lead chromate caused 20 and 48 chromatid exchanges in 100 metaphases for XRCC3-deficient and RAD51C-deficient cells compared to 0 chromatid exchanges in 100 metaphases for the wildtype (p value: <0.0057 , <0.0001 , respectively). Complemented cells of each mutant were restored to wild-type levels (p value: <0.9203 , <1.0000 , respectively).

4. Discussion

Chromosome instability (CIN) is a hallmark of chromium-associated lung cancer. However, the mechanisms of these carcinogenic rearrangements involved, and the cellular DNA damage signaling and repair pathways acting to prevent such mutagenic events are not well defined [3]. Cr(VI) is a well-established human lung carcinogen and previous studies show that Cr(VI) chromium is most potent in particulate form. Particulate Cr(VI) induces DNA strand breaks, Cr-DNA adducts, Cr-DNA crosslinks and mutation to 6-thioguanine resistance in diploid human fibroblasts [8,14–17,12,35,36], however how these lesions are repaired and their relationship to Cr(VI)-induced CIN are uncertain.

In this study we show that the homologous recombination repair pathway plays a role in protecting cells from particulate Cr(VI)-induced CIN. Specifically, the Rad51C paralogs XRCC3 and RAD51C function to prevent simple and complex chromosome aberrations induced by particulate Cr(VI). Deficiency in either of these repair proteins also increased the Cr(VI)-induced frequency and total amount of chromosomal damage and caused a dramatic shift in spectrum of aberrations. This conclusion is supported by published data showing that Cr(VI)-induced DNA double strand breaks are preferentially formed after the S-phase of the cell cycle, likely during DNA replication, when HR repair is the most active [37–39]. Importantly, the role of HR in dealing with Cr(VI)-associated lesions is consistent with our recent data showing that a major alternative pathway for the repair of double strand breaks, NHEJ, does not protect cells from particulate Cr(VI)-induced CIN [20]. These data are the first

to consider genes involved in HR repair and Cr(VI)-induced chromosome damage. They are consistent with previous reports of functional polymorphisms in XRCC3, which correlate with increased chromosome damage in metal-exposed workers and with increased risk of lung cancer [40–41].

Both genes participate in the repair of DNA double strand breaks. XRCC3 is thought to participate in the resolvase step and RAD51C in both the resolvase step and in presynapsis of HR repair [24–25]. Thus, our data strongly suggest that DNA double strand breaks, associated with DNA replication, underlie Cr(VI)-induced chromatid aberrations, particularly chromatid exchanges. Generation of chromatid exchanges may lead to an increase in chromosome translocations, a hallmark of lung cancer [42–44]. Other published data suggest that Cr(VI)-induced DNA double strand breaks probably result from replication fork collapse during the repair of single strand breaks, crosslinks or adducts [26], consistent with our previous report showing that a deficiency in single strand breaks also leads to more complex chromosomal aberrations [33]. Any unrepaired double strand breaks then manifest as chromatid aberrations and exchanges in mitosis. The proximate genotoxin for particulate and soluble Cr(VI) is the same (chromate anion) and thus, the mechanism of formation of the double strand break should also be the same though it has not yet been demonstrated [45].

We also found that both XRCC3- and RAD51C-deficient cells are more sensitive to the cytotoxic effects of chromium. These data are consistent with our previous reports showing that cells deficient in Ku80, FANCG and XRCC1 are also sensitive to the cytotoxic effects of particulate Cr(VI) [33,28,20]. They are also consistent with a previous study of soluble chromate showing that XRCC3- and BRCA2-deficient cells were more sensitive to its cytotoxicity [23]. Considered altogether they indicate that after particulate Cr(VI) exposure the DNA repair machinery can interact with cell death pathways and delay apoptosis to allow time for repair.

In this study, and in previous reports using DNA-repair defective cells, we find that DNA repair-deficient cells, including those deficient in Ku80, XRCC1, and FANCG, exhibited large differences in the levels of intracellular genotoxic chemicals compared to their parental and gene complemented control cells [33,20,28]. Such differences in uptake can potentially reverse the conclusions of the relative sensitivities of a cell strain to an agent based only on administered dose. These data observations are significant as recent studies consider the repair of chemically-induced lesions including mitomycin C, psoralen and Cr(VI), but fail to account for differences in chemical uptake. A recent study measuring the Cr(VI)-induced toxicity of HR repair-deficient cells to the parent cells failed to consider the differential uptake of the compound among the cells in the study, thus potentially compromising their conclusions [23].

In summary, we show for the first time a role for the HR repair associated genes, XRCC3 and RAD51C in protecting cells from particulate Cr(VI)-induced CIN. Thus, implicating HR repair to be the primary repair pathway for resolving Cr(VI)-induced DNA double strand breaks. Further work will focus on understanding the link between HR repair in prevent Cr(VI)-induced neoplastic transformation and translocations.

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References

1. Masuda A, Takahashi T. Chromosome instability in human lung cancers: possible underlying mechanisms and potential consequences in the pathogenesis. *Oncogene* 2002;21:6884–6897. [PubMed: 12362271]
2. Nakamura H, Saji H, Idiris A, Kawasaki N, Hosaka M, Ogata A, Saijo T, Kato H. Chromosomal instability detected by fluorescence *in situ* hybridization in surgical specimens in non-small cell lung cancer is associated with poor survival. *Clin Cancer Res* 2003;9:2294–2299. [PubMed: 12796398]
3. Hirose T, Kondo K, Takahashi Y, Ishikura H, Fujino H, Tsuyuguchi M, Hashimoto M, Yokose T, Mukai K, Kodama T, Monden Y. Frequent microsatellite instability in lung cancer from chromate-exposed workers. *Mol Carcin* 2002;33:172–180.
4. Takahashi Y, Kondo K, Hirose T, Nakagawa H, Tsuyuguchi M, Hashimoto M, Sano T, Ochiai A, Monden Y. Microsatellite instability and protein expression of the DNA mismatch repair gene, hMLH1, of lung cancer in chromate-exposed workers. *Mol Carcinog* 2005;42:150–158. [PubMed: 15605365]
5. IARC. Monographs on the evaluations of carcinogenic risks to humans. 49. 1990. Chromium, nickel and welding; p. 1-648.
6. Levy LS, Martin PA, Bidstrup PL. Investigation of the potential carcinogenicity of a range of chromium containing materials on rat lung. *Br J Ind Med* 1986;43:243–256. [PubMed: 3964573]
7. Patierno SR, Banh D, Landolph JR. Transformation of C3H/10T1/2 mouse embryo cells to focus formation and anchorage independence by insoluble lead chromate but not soluble calcium chromate: relationship to mutagenesis and internalization of lead chromate particles. *Cancer Res* 1988;48:5280–5288. [PubMed: 3409252]
8. Wise JP, Leonard JC, Patierno SR. Clastogenicity of lead chromate particles in hamster and human cells. *Mutat Res* 1992;278:69–79. [PubMed: 1370121]
9. Wise SS, Holmes AL, Ketterer ME, Hartsock WJ, Fomchenko E, Katsifis S, Thompson WD, Wise JP Sr. Chromium is the proximate clastogenic species for lead chromate-induced clastogenicity in human bronchial cells. *Mutat Res* 2004;560:79–89. [PubMed: 15099827]
10. Singh J, Pritchard DE, Carlisle DL, Mclean JA, Montaser A, Orenstein JM, Patierno SR. Internalization of carcinogenic lead chromate particles by cultured normal human lung epithelial cells: formations of intracellular lead-inclusion bodies and induction of apoptosis. *Toxicol Appl Pharmacol* 1999;161:240–248. [PubMed: 10620481]
11. Wise JP, Orenstein JM, Patierno SR. Inhibition of lead chromate clastogenesis by ascorbate: relationship to particle dissolution and uptake. *Carcinogenesis* 1993;14:429–434. [PubMed: 8453719]
12. Wise JP Sr, Wise SS, Little JE. The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cells. *Mutat Res* 2002;517:221–229. [PubMed: 12034323]
13. Xu J, Wise JP, Patierno SR. DNA damage induced by carcinogenic lead chromate particles in cultured mammalian cells. *Mutat Res* 1992;280:129–136. [PubMed: 1378537]
14. Biedermann KA, Landolph JR. Induction of Anchorage Independence in Human Diploid Foreskin Fibroblasts by Carcinogenic Metal Salts. *Cancer Res* 1987;47:3815–3823. [PubMed: 3594439]
15. Biedermann KA, Landolph JR. Role of Valence State and Solubility of Chromium Compounds on Induction of Cytotoxicity, Mutagenesis, and Anchorage Independence in Diploid Human Fibroblasts. *Cancer Res* 1990;50:7835–7842. [PubMed: 2253225]
16. Sugiyama M, Ando A, Ogura R. Effects of vitamin E on survival, glutathione reductase and formation of chromium (V) in Chinese hamster V-79 cells treated with sodium chromate (VI). *Carcinogenesis* 1989;10:737–741. [PubMed: 2649268]
17. Sugiyama M, Ando A, Nakao K, Ueta H, Hidaka T, Ogura R. Influence of vitamin B₂ on formation of chromium (V), alkali labile sites, and lethality of sodium chromate (VI) in Chinese hamster V-79 cells. *Cancer Res* 1989;49:6180–6184. [PubMed: 2553247]
18. Sugiyama M, Lin X, Costa M. Protective effect of vitamin E against chromosomal aberrations and mutation induced by sodium chromate in Chinese hamster V79 cells. *Mutat Res* 1991;260:19–25. [PubMed: 2027337]

19. Sugiyama M, Tsuzuki K, Lin X, Costa M. Potentiation of sodium chromate (VI)-induced chromosomal aberrations and mutation by vitamin B₂ in Chinese hamster V79 cells. *Mutat Res* 1992;283:211–214. [PubMed: 1383790]
20. Camrye E, Wise SS, Milligan P, Gordon N, Goodale B, Stackpole M, Patzlaff N, Aboueissa A, Wise JP Sr. Ku80 Deficiency Does Not Affect Particulate Chromate-Induced Chromosome Damage and Cytotoxicity in Chinese Hamster Ovary Cells. Submitted to *Tox Sci*.
21. Wyman C, Ristic D, Kanaar R. Homologous recombination-mediated double-strand break repair. *DNA Repair* 2004;3:827–833. [PubMed: 15279767]
22. Rodrigue A, Lafrance M, Gauthier M, McDonald D, Hendzel M, West S, Jasin M, Masson J. Interplay between human DNA repair proteins at a unique double-strand break in vivo. *EMBO Journal* 2006;25:222–231. [PubMed: 16395335]
23. Bryant HE, Ying S, Helleday T. Homologous recombination is involved in repair of chromium-induced DNA damage in mammalian cells. *Mutat Res* 2006;599:116–23. [PubMed: 16564059]
24. Liu Y, Masson JY, Shah R, O'Regan P, West SC. RAD51C Is Required for Holliday Junction Processing in Mammalian Cells. *Science* 2004;303:243–246. [PubMed: 14716019]
25. Liu Y, Tarsounas M, O'Regan P, West SC. Role of RAD51C and XRCC3 in Genetic Recombination and DNA Repair. *J Biol Chem* 2007;282:1973–1979. [PubMed: 17114795]
26. Fuller LF, Painter RB. A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. *Mutat Res* 1998;193:109–121. [PubMed: 3347204]
27. Yamada NA, Hinz JM, Kopf VL, Segalle KD, Thompson LH. XRCC3 ATPase activity is required for normal XRCC3-RAD51C complex dynamics and homologous recombination. *J Biol Chem* 2004;279:23250–23254. [PubMed: 15037616]
28. Savery LC, Grlickova-Duzevick E, Wise SS, Thompson WD, Hinz JM, Thompson LH, Wise JP Sr. Role of the Fancg gene in protecting cells from particulate chromate-induced chromosome instability. *Mutat Res* 2007;626:120–127. [PubMed: 17097336]
29. Jones NJ, Cox R, Thacker J. Isolation and cross-sensitivity of X-ray-sensitive mutants of V79-4 hamster cells. *Mutat Res* 1987;183:279–86. [PubMed: 3106801]
30. French CA, Masson JY, Griffin CS, O'Regan P, West SC, Thacker J. Role of mammalian RAD51L2 (RAD51C) in recombination and genetic stability. *J Biol Chem* 2002;277:19322–19330. [PubMed: 11912211]
31. Wise JP, Leonard JC, Patierno SR. Clastogenesis by particulate PbCrO₄ in hamster and human cells. *Mutat Res* 1992;278:69–79. [PubMed: 1370121]
32. Grlickova-Duzevick E, Wise SS, Munroe RC, Thompson WD, Wise JP Sr. XRCC1 protects cells from chromate-induced chromosome damage, but does not affect cytotoxicity. *Mutat Res* 2006;610:31–37. [PubMed: 16904935]
33. Grlickova-Duzevick E, Wise SS, Munroe RC, Thompson WD, Wise JP Sr. XRCC1 Protects Against Particulate Chromate-Induced Chromosome Damage and Cytotoxicity in Chinese Hamster Ovary Cells. *Toxicol Sci* 2006;92:96–102. [PubMed: 16597656]
34. SAS Institute, Inc. SAS/STAT 9.1 User's Guide. Cary NC: SAS Institute, Inc; 2004.
35. Wise SS, Elmore LW, Holt SE, Little JE, Bryant BH, Wise JP Sr. Telomerase-mediated lifespan extension of human bronchial cells does not affect hexavalent chromium-induced cytotoxicity or genotoxicity. *Mol Cell Biochem* 2004a;255:103–111. [PubMed: 14971651]
36. Holmes L, Wise SS, Sandwick SJ, Lingle WL, Negron VC, Thompson WD, Wise JP Sr. Chronic Exposure to Lead Chromate Causes Centrosome Abnormalities and Aneuploidy in Human Lung Cells. *Cancer Res* 2006;66(8):4041–4048. [PubMed: 16618723]
37. Ha L, Ceryak S, Patierno SR. Generation of S phase-dependent DNA double-strand breaks by Cr(VI) exposure: involvement of ATM in Cr(VI) induction of gamma-H2AX. *Carcinogenesis* 2004;25:2265–2274. [PubMed: 15284180]
38. van den Bosch M, Lohman PH, Pastink A. DNA double-strand break repair by homologous recombination. *Biol Chem* 2002;383:873–892. [PubMed: 12222678]
39. Ferreira MG, Cooper JP. Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. *Genes Dev* 2004;18:2249–2254. [PubMed: 15371339]

40. Mateuca R, Aka PV, De Boeck M, Hauspie R, Kirsch-Volders M, Lison D. Influence of hOGG1, XRCC1 and XRCC3 genotypes on biomarkers of genotoxicity in workers exposed to cobalt or hard metal dusts. *Toxicol Letters* 2005;156:277–288.
41. Jacobsen NR, Raaschou-Nielsen O, Nexø B, Wallin H, Overvad K, Tjønneland A, Vogel U. XRCC3 polymorphisms and risk of lung cancer. *Cancer Letters* 2004;213:67–72. [PubMed: 15312685]
42. Cleary ML. Oncogenic conversion of transcription factors by chromosomal translocations. *Cell* 1991;66:619–622. [PubMed: 1878967]
43. Croce CM. Chromosome translocations and human cancer. *Cancer Res* 1986;46:6019–6023. [PubMed: 3536075]
44. Testa JR. Chromosome translocations in human cancer. *Cell Growth Differ* 1990;1:97–101. [PubMed: 2085464]
45. Xie H, Wise SS, Holmes AL, Xu B, Wakeman TP, Pelsue SC, Singh NP, Wise JP Sr. Carcinogenic lead chromate induces DNA double-strand breaks in human lung cells. *Mutat Res* 2005;586:160–172. [PubMed: 16112599]

Figure 1A

Figure 1B

Figure 1. Intracellular Ion Uptake of Chromium

Cells deficient in XRCC3 have less Cr uptake whereas cells deficient in RAD51C have more Cr uptake than wildtype and cDNA-complemented cells. A) Intracellular chromium ion concentrations of AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented). B) Intracellular chromium ion concentrations of V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6 (RAD51C-complemented). Data represent the average of at least 3 independent experiments $\pm$ standard error of the mean

Figure 2A

Figure 2B

Figure 2C

Figure 2D

Figure 2. Cytotoxic Effects of Lead Chromate

Cells deficient in XRCC3 and RAD51C are more sensitive to the cytotoxic effects of particulate Cr(VI). A) The cytotoxicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on administered concentration. B) The cytotoxicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on the measured intracellular Cr concentration. C) The cytotoxicity of lead chromate in V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6 (RAD51C-complemented) based on the measured administered concentration. D) The cytotoxicity of lead chromate in V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6

(RAD51C-complemented) based on intracellular Cr concentration. Data represent the average of at least 3 independent experiments $\pm$ standard error of the mean.

Figure 3A

Figure 3B

Figure 3C

Figure 3D

Figure 3. Percent of Metaphases with Damaged Chromosomes

Lead chromate induced a greater percentage of metaphases with chromosomal damage in cells deficient in XRCC3 and RAD51C. A) The clastogenicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on administered concentration. B) The clastogenicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on intracellular Cr concentration. C) The clastogenicity of lead chromate in V79 (wildtype), irs3 (RAD51C deficient) and irs3#6 (RAD51C-complemented) based on administered concentration. D) The clastogenicity of lead chromate in V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6 (RAD51C-complemented)

based on intracellular Cr concentration. Data represent the average of at least 3 independent experiments $\pm$ standard error of the mean. Background (control) levels have been subtracted for each data point, AA8 control 1.7 ± 0.9 , irs1SF control 7.6 ± 2.3 , 1SFwt8 control 3.3 ± 2.4 , V79 control 2.3 ± 1.3 , irs3 control 10 ± 2.6 , irs3#6 control 2.6 ± 1.2

Figure 4A

Figure 4B

Figure 4C

Figure 4D

Figure 4. Total Metaphases with Damaged Chromosomes

Lead chromate induced a greater amount of total chromosome damage in 100 metaphases of cells deficient in XRCC3 and RAD51C. A) The clastogenicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on administered concentration. B) The clastogenicity of lead chromate in AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented) based on intracellular Cr concentration. C) The clastogenicity of lead chromate in V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6 (RAD51C-complemented) based on administered concentration. D) The clastogenicity of lead chromate in V79 (wildtype), irs3 (RAD51C-deficient) and irs3#6 (RAD51C-complemented) based on intracellular Cr concentration. Data represent the average of at least 3 independent experiments $\pm$ standard error of the mean. Background (control) levels have been subtracted for each data point. AA8 control 2.0 ± 1.0 , irs1SF control 8.3 ± 3.0 , 1SFwt8 control 3.6 ± 2.7 , V79 control 2.3 ± 1.3 , irs3 control 11 ± 2.9 , irs3#6 control 2.6 ± 1.2 .

Figure 5A

Figure 5B

Figure 5. Spectrum of Chromosome Damage

Total chromatid lesions and exchanges induced by lead chromate in 100 metaphase cells deficient in XRCC3 and RAD51C. A) The spectrum of damage for AA8 (wildtype), irs1SF (XRCC3-deficient) and 1SFwt8 (XRCC3-complemented). B) The spectrum of damage for V79 (wildtype), irs3 (RAD51C deficient), irs3#6 (RAD51C-complemented). Data represent the average of at least 3 independent experiments $\pm$ standard error of the mean and background (control) has been removed. For chromatid lesions: AA8 control 1.7 ± 0.9 , irs1SF control 5.3 ± 2.0 , 1SFwt8 control 2.6 ± 2.7 , V79 control 2.3 ± 1.3 , irs3 control 7.3 ± 1.8 , irs3#6 control 1.6 ± 1.2 . For chromatid exchanges: AA8 control 0, irs1SF control 3.0 ± 1.2 , 1SFwt8 control 0, V79 control 0, irs3 control 2.6 ± 1.8 , irs3#6 control 0.

Table 1
Chinese Hamster Cell Lines Used to Study Homologous Repair

Cell Phenotype	RAD51C	XRCC3
Wild-type cell	V79	AA8
Gene Deficient	irs3	irs1SF
Gene Restored	irs3#6	1SFwt8