Cr (VI) Influences Inflammatory Cytokines in Human Lung Cells

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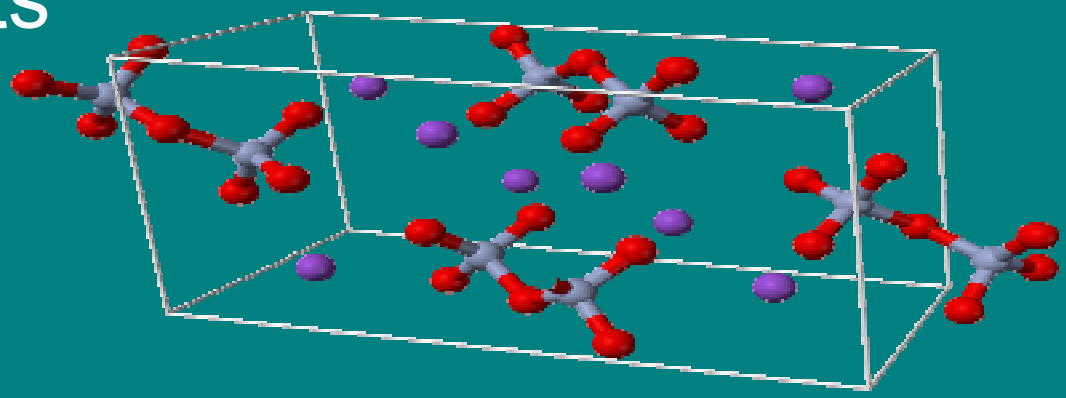
Overview

- What is Cr (VI)?
- Where is Cr (VI) found?
- What the effects on human health?
- How does it interact with lung cells?
- How does Cr (VI) influence inflammation?
- Research Results



Cr (III) vs. Cr (VI)

- Cr (III) is a dietary mineral required in trace amounts for glucose metabolism
- Cr (VI) is mutagenic when inhaled
  - lethal ingested dose = 2.373 grams

Cr (III) ≠ Cr (VI)

Hexavalent Chromium Cr (VI)

- Cr (VI) compounds contain elemental chromium in the 6th oxidation state.
- It has widespread use in industries such as:
  - alloy production
  - automobile manufacturing
  - battery factories
  - cement industry
  - electroplating
  - foundry work
  - glass making
  - scientific laboratories
  - leather tannery
  - metal workers
  - miners
  - painters
  - paper manufacturing
  - photography

(Dweck et al 2005, Michaels et al 2006, Vitale et al 1997)

Cr (VI) Toxicity

- Adverse Health Effects
  - Skin irritation and ulceration (contact dermatitis)
  - Perforated nasal septum
  - Nasal bleeding
  - Conjunctivitis

Cr (VI) Carcinogenicity

"There is *sufficient evidence* in humans for the carcinogenicity of chromium[VI] compounds as encountered in the chromate production, chromate pigment production and chromium plating industries."

- World Health Organization, International Agency for Research on Cancer (Volume 49, 1997)

Epidemiological Evidence

- Several cohort studies of industrial workers exposed to Cr (VI) airborne particles show an increased risk for lung cancer.

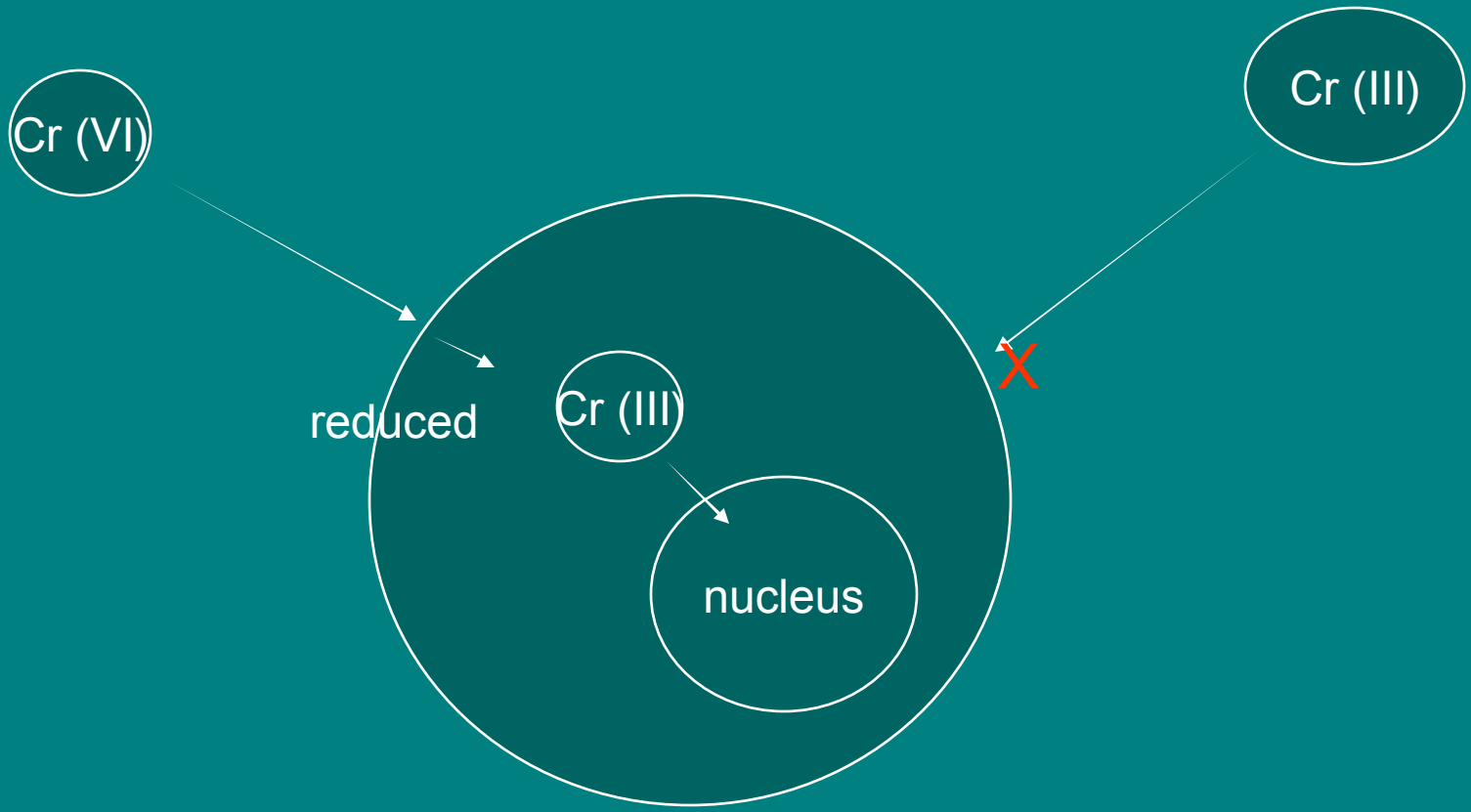
  Gibb et al. 2000

  Park et al. 2004

  Park and Stayner 2006,

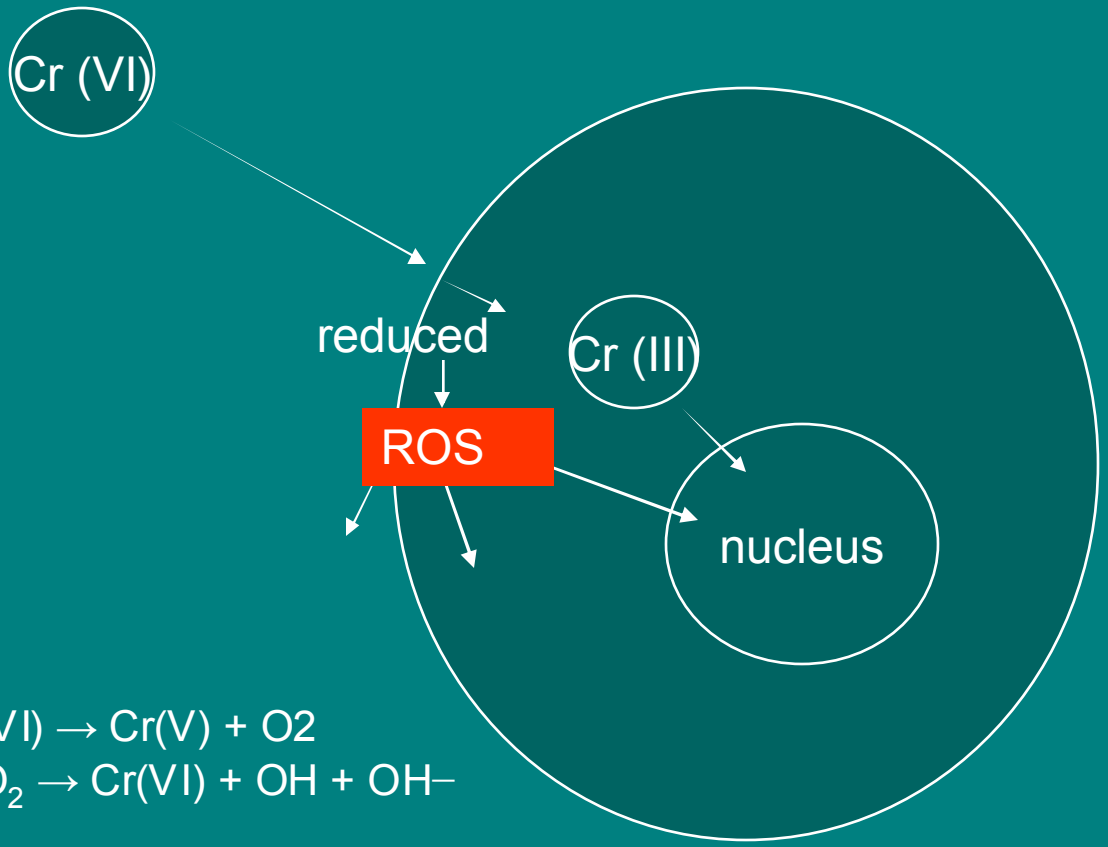
  Birk et al. 2006

Transport into the Cell



Production of ROS

- Reactive Oxygen Species (ROS)



1)$O2^{\cdot -}$ + Cr(VI) → Cr(V) + O2
2)Cr(V)+ H_2O_2 → Cr(VI) + OH + OH^-
Overall:
3)$O2^{\cdot -}$ + H2O2 → O2 + •OH + OH^-
(Leonard et al. 2004)

Production of ROS Can Lead To:

- DNA damage and further inflammation (Babbar and Casero 2006)
- Increased activity of the P53 cell cycle checkpoint defense (Wang et al. 2000)
- Dissipation of plasma and mitochondria membrane potential in Chinese hamster lung cells (Xie et al. 2001)

Chromosome & DNA Damage

- Chromosome damage
  - Spindle assembly
  - Centrosome dysfunction
  - Aneuploidy

    (Holmes et al., 2006; Wise et al 2006)
- DNA Level
  - Excess double strand breaks
  - DNA-protein cross links (Xie et al., 2005; Liu et al., 2001)
  - specific Cr (III)-DNA adducts and Cr (III)-histidine-DNA adducts in p53 gene sequence of DNA fragments (Arakawa et al., 2006)

Cr (VI) Induced Inflammation

- Cr (VI) exposure up-regulated expression of transcription factors NF-κB and AP-1, important inflammatory pathway genes

  (Leonard et al., 2004).

- *In vitro* models show the release of IL-6 and IL-8 was initiated by chromium (VI)

  (Pascal et al., 2004).

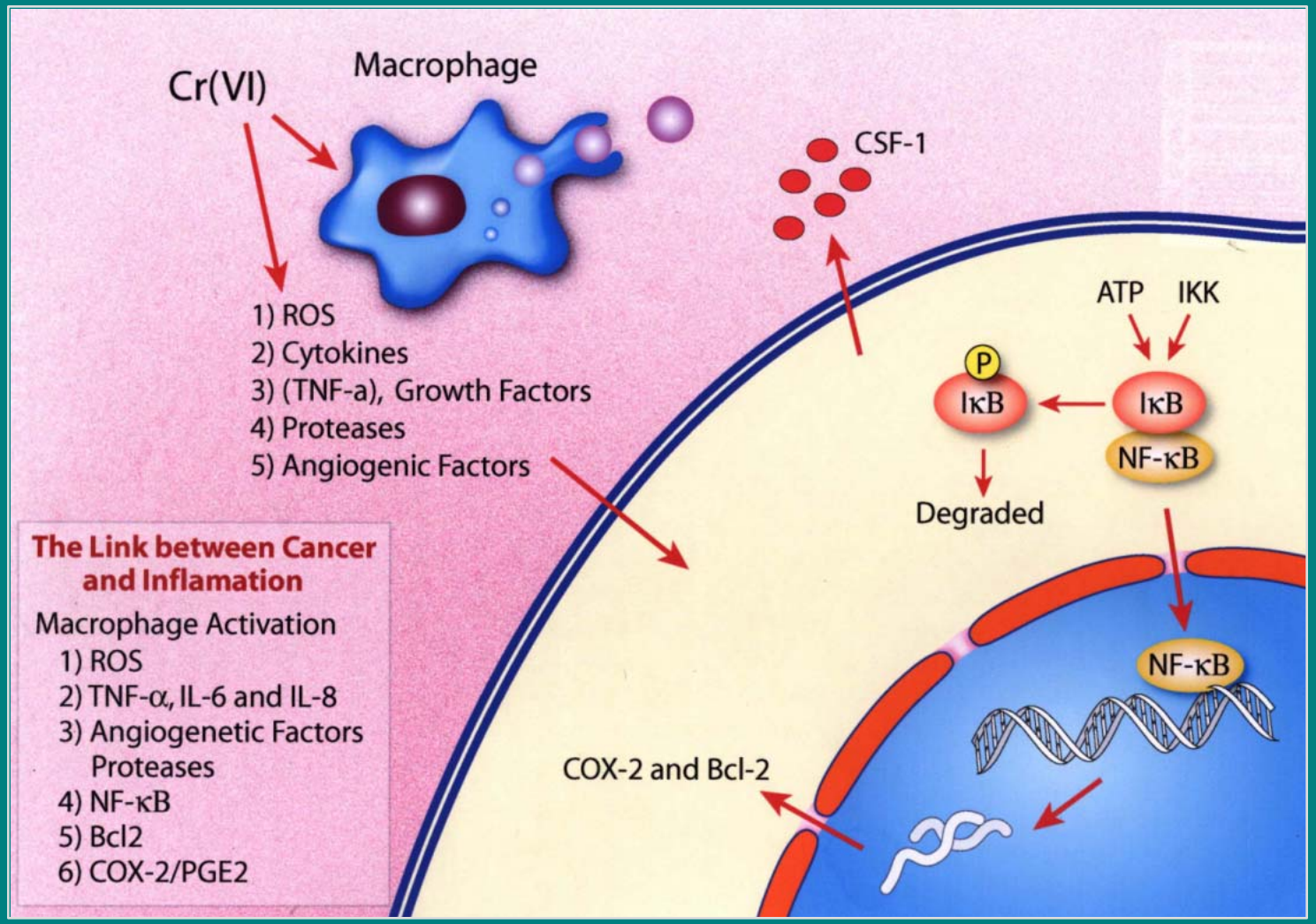
Affymetrix® Gene Chip Studies

Lung cell exposure to Cr (VI) resulted in altered gene expression of:

- DNA repair
- transcription
- energy metabolism
- calcium mobilization
- signal transduction
- apoptosis
- redox stress
- cell cycle
- cell adhesion
- protein synthesis

(Pritchard et al., 2005; Ye et al., 2001)

Proposed Pathway



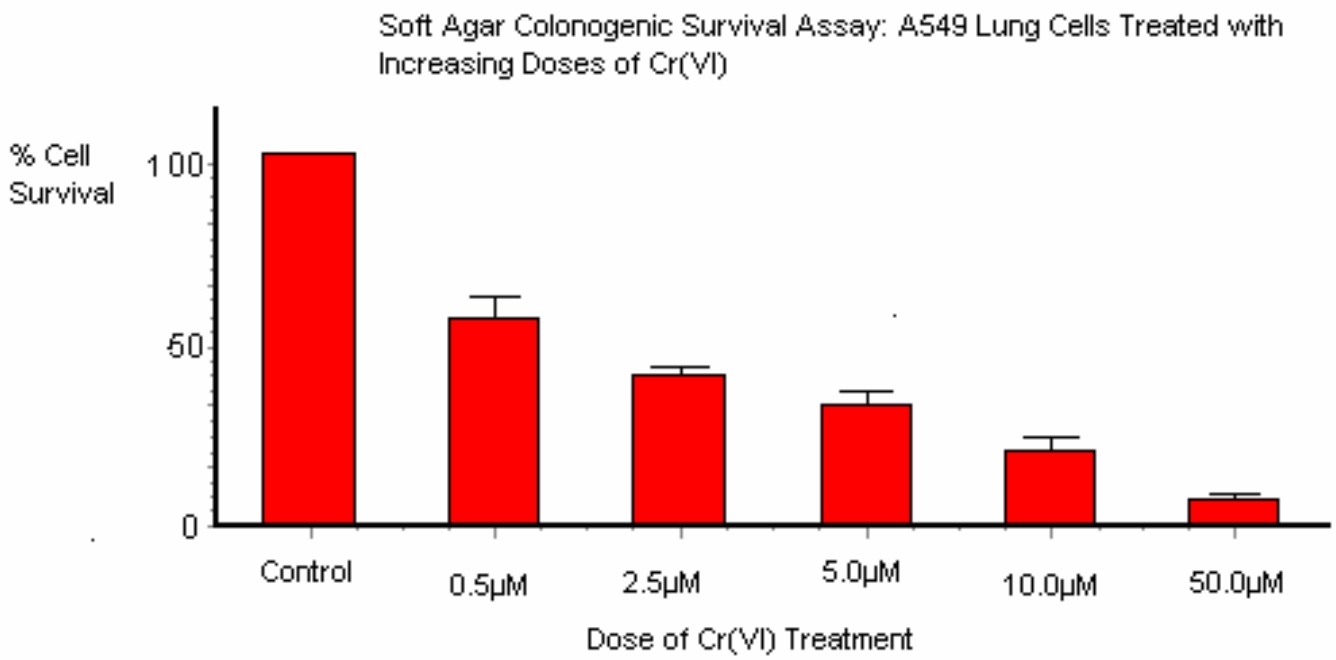
Schematic model predicting the possible role of mediators of inflammation in response to Cr (VI) and activation of NF-kB/Ap-1 pathways involved in COX-2 expression (Modified from Marx J. Science 2004; 306: 966-83)

Research Objectives

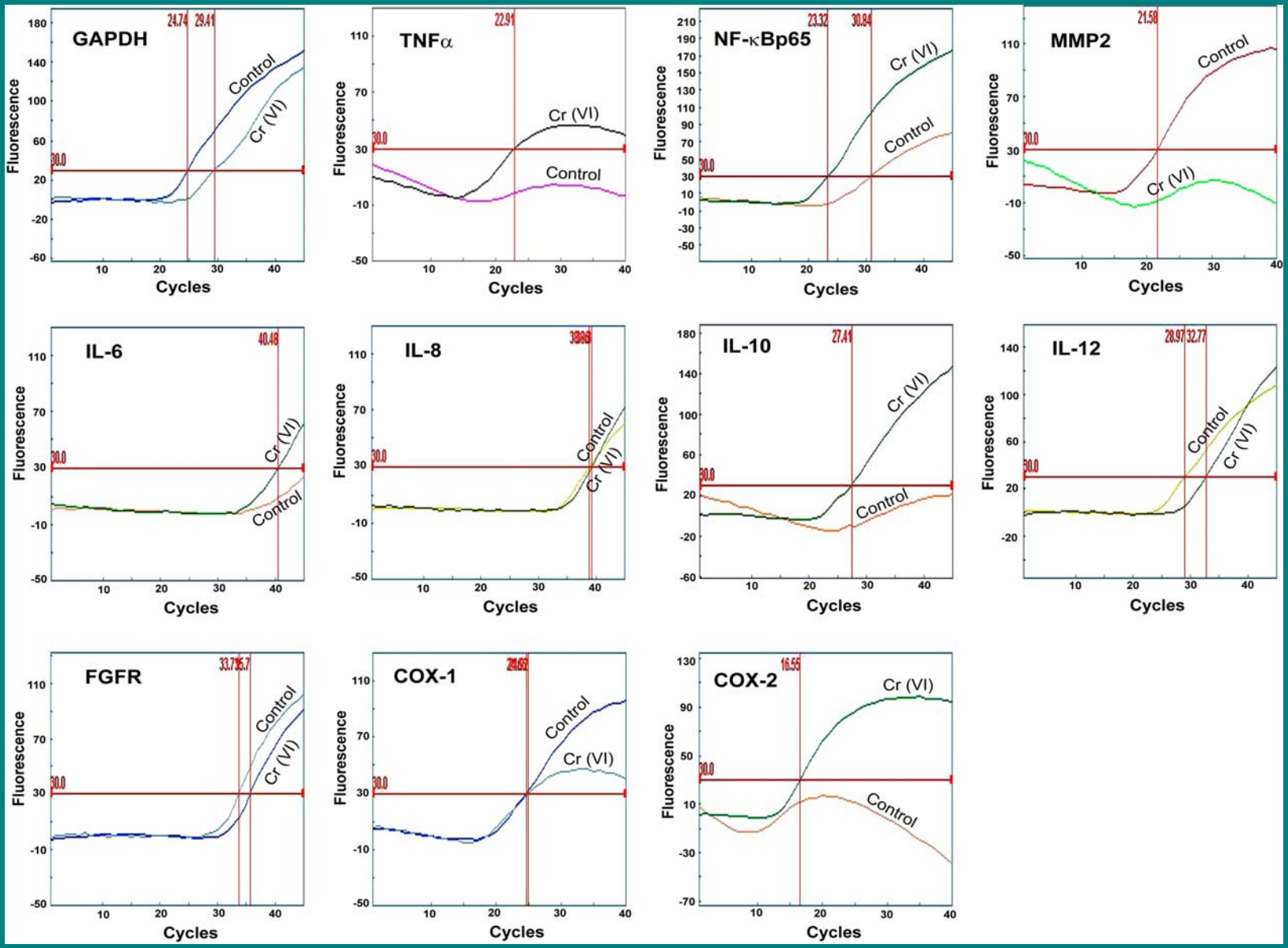
- Occupation related epidemiological reports are indicating a strong link between hexavalent chromium [Cr (VI)] exposure and lung diseases including cancer.

- Our goal is to investigate the mechanism(s) of Cr (VI) mediated inflammation and the risk for lung cancer.

Soft Agar Clonogenic Cell Survival Toxicity Assay



The Soft Agar Clonogenic Survival Assay of A549 Lung cells treated with increasing doses of Cr (VI). The Student t-test gives us a value of <.05 for treatment groups 0.5-50 µMol compared to the control group. Data is reported as percent of the mean (n=3) +/- SD



Real time PCR-analysis. Total RNA extracted from chromium exposed cells were used to detect the transcription (mRNA) levels of mediators of inflammation. Human lung cancer (A 549) cells was treated with 2.5 μM Cr (VI) for 48 h. RT-PCR amplification was carried out using gene specific primers. Amplification of GAPDH was used as the internal control. Note: Increase in the amplification cycles indicate lower amount of mRNA.

Unchanged Gene Expression

- GAPDH (**Glyceraldehyde 3-phosphate dehydrogenase**)
- IL-12 (Interleukin 12)
- IL-8 (Interleukin 8)
- FGFR (**fibroblast growth factor receptors**)

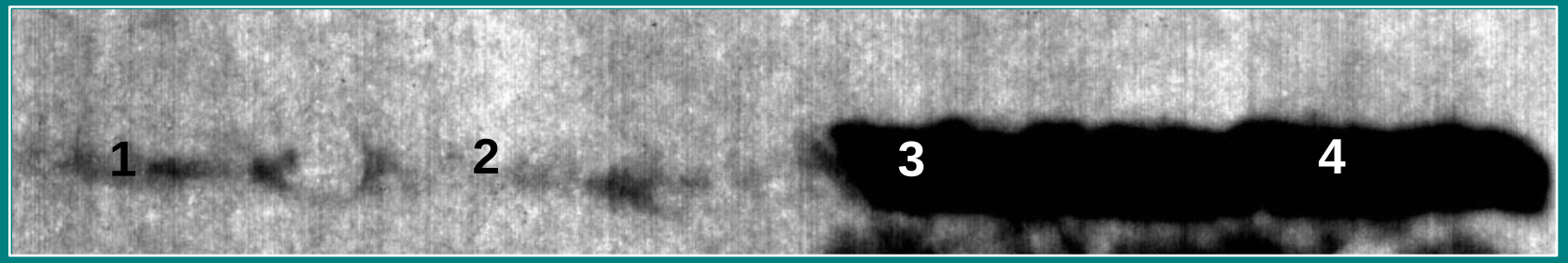
Under Expressed Gene

- COX-1 (cyclooxygenase-1)
- MMP2 (matrix metalloproteinases 2)

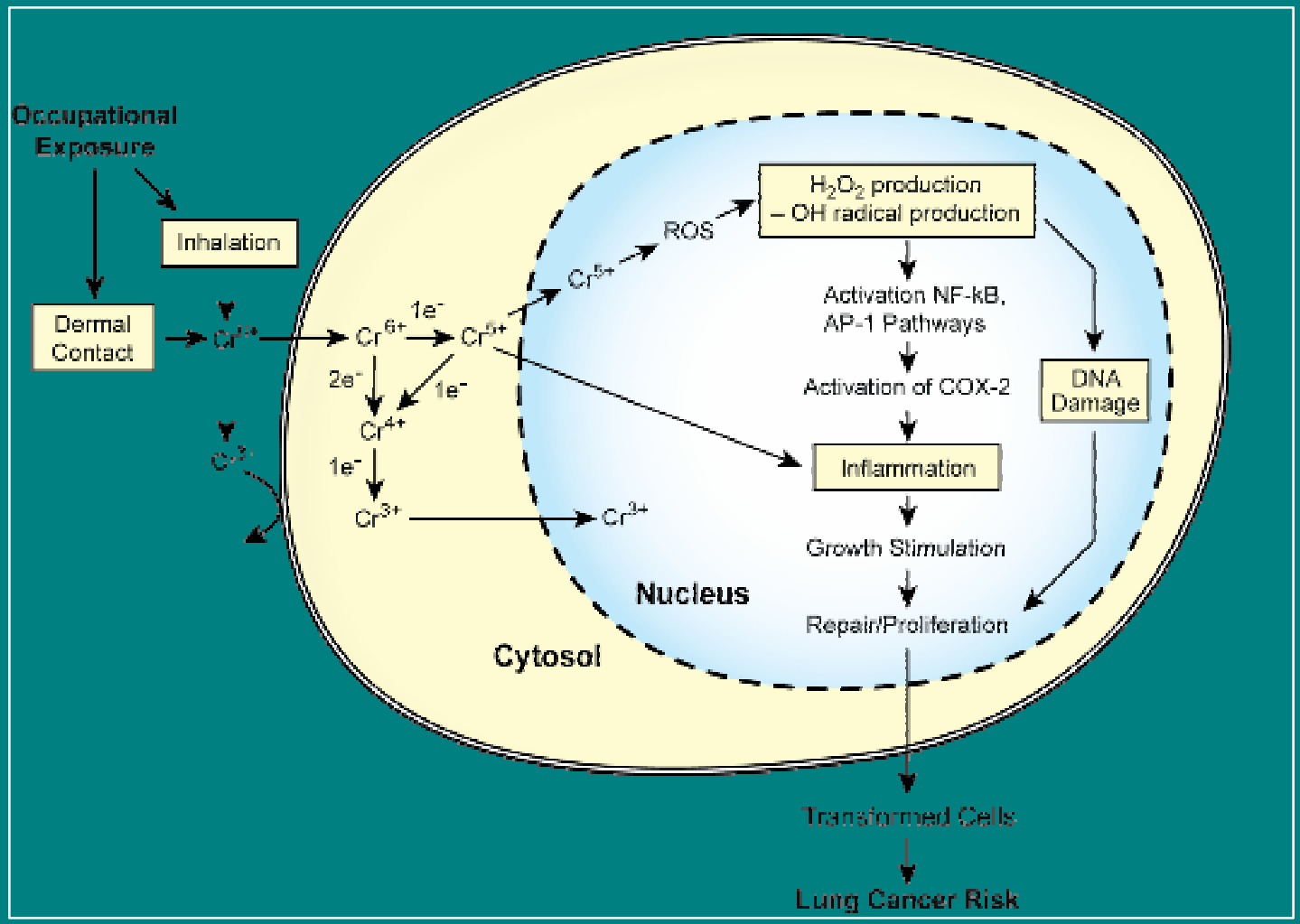
Over Expressed Genes

- IL-6 (Interleukin-6)
- IL-10 (Interleukin-10)
- COX-2 (cyclooxygenase-2)
- TNF-α (tumor necrosis factor-α)
- NF-κ B (nuclear factor- kappa B)

NF-κBp65 protein western blot



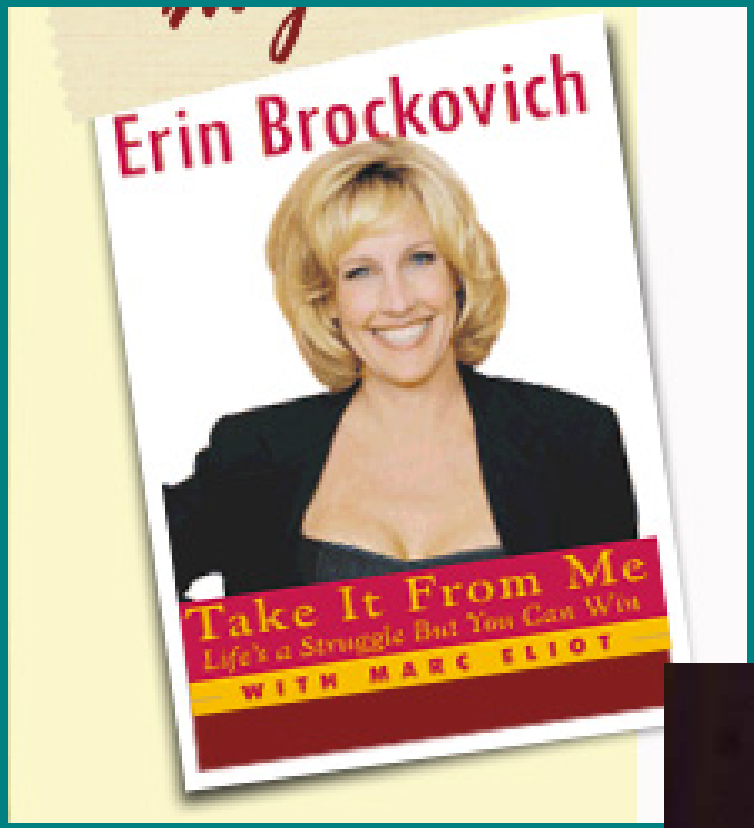
NF-κBp65 protein western blot. A549 lung cells treated with Cr(VI) in combination with PMA showed enhanced expression of the NF-κBp65 protein than untreated cells. Exposure time 5 minutes. **1,** Untreated A549 cells; **2,** untreated A549 cells**; 3,** Treated with 2.5 μM Cr(VI); **4,**Treated with 2.5 μM Cr(VI).



Schematic model showing the Chromium(VI) mediated inflammatory pathways:
- Cr(VI) particles within the cell can activate transcription factors such as the NF-κB and AP-1 involved in mediating inflammation. Activation of this inflammatory pathway could mediate several signaling molecules involved in inflammation within the mammalian system. In this illustration, we predict the role of Cr(VI) in initiating several cascade of events starting from the macrophage activation leading to production of ROS, TNF-α, IL-6/IL-8, growth factors and angiogenic factors that trigger the transcription of nuclear NF-κB and cyclooxygenases. This model predicts the overall activation of the mediators of inflammation among workers who are chronically exposed to Cr(VI) and develop the risk for lung cancer.

Results

1. Enhanced gene expression of IL-6, IL-10, Cox-2, NF-κB and TNF-alpha in chromium exposed cells indicate the activation of pro-inflammatory pathways
2. Enhanced production of the NF-κB65 protein in chromium exposed cells further supports its role in inflammation
3. Findings suggests investigating anti-inflammatory agents in prevention strategies.



Key Note speaker at the APHA Annual Meeting in 2004

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