Title

The ETS domain-containing hematopoietic transcription factor PU.1 mediates the induction of arachidonate 5-lipoxygenase by multi-walled carbon nanotubes in macrophages *in vitro*_dataset

Introduction

Fibrogenic multi-walled carbon nanotubes (MWCNTs) polarize macrophages to M1 cells with elevated production of proinflammatory lipid mediators (LMs) to boost acute inflammation. Here we examined the mechanism by which MWCNTs (Mitsui-7) induce arachidonate 5-lypoxygenase (Alox5) critical for production of chemotactic LM leukotriene B4 (LTB4) in murine and human macrophages.

Methods Collection

1. Particle preparation

- Stock solutions of MWCNTs (Mitsui-7) and carbon black (CB) were prepared in a control medium (CM) [Dulbecco's modified eagle's medium (DMEM) with 1% fetal bovine serum (FBS)] at a concentration of 2 mg/ml through vortexing and by sonication and were further diluted with the culture media and sonicated immediately before use.

2. Cell culture, polarization, and treatment

- J774A.1 murine monocyte/macrophage cells grown in DMEM with 10% fetal bovine serum were seeded at 5 x 10^5 cells/ml in DMEM with 3% FBS for 1 day and then treated with other reagents [MWCNTs (2.5 or 10 μg/ml) or CB (2.5 or 10 μg/ml)] for 1 day.
- Interferon-γ (20 ng/ml) plus lipopolysaccharides (100 ng/ml) was used as a positive control and control media (DMEM media with 1% FBS) were used as a negative control.
- Human monocytes (2.5 × 10^6 cells/well) isolated from the whole peripheral blood were grown in the ImmunoCult-SF macrophage medium supplemented with M-CSF at 50 ng/mL for 4 days to induce their differentiation into macrophages (human monocyte derived macrophages, HMDMs) and used for cell treatment like above.

3. Quantitative real-time PCR (RT-qPCR)

- Detection and quantification of mouse *Alox5* or *β-actin* and human *ALOX5* or *β-ACTIN* at mRNA level.

4. Luciferase reporter assay

- Detection and quantification of mouse *Alox5* promoter activity using luciferase reporter plasmids carrying various lengths of the promoter region of the mouse *Alox5* gene.
- The luciferase activity was measured by using a GloMax 20/20 luminometer and were normalized to the β-gal intensity of the same samples for correct for variations.

5. Chromatin immunoprecipitation assay (ChIP)

- ChIP assay was performed to detect the transcription factor PU.1 binding to the promoter region of the mouse *Alox5* gene using a Magnify chromatin immunoprecipitation kit and anti-PU.1 rabbit monoclonal antibody.

6. PU.1 knockdown by short hairpin RNA (shRNA)

- To knockdown PU.1 gene expression in macrophages, mouse PU.1-specific short hairpin (shRNA) as RNA interference was introduced into macrophages and incubated for 2 days and then cells were used for RT-qPCR and immunoblotting analysis.

7. Immunoblotting

- Detection and quantification of Alox5, PU.1, or β-actin at protein level.
- Images were scanned using HP scanjet and were used to quantify each band with ImageJ software.

8. Enzyme-linked immunosorbent assay (ELISA)

- Production of proinflammatory lipid mediators (LTB4, PGE2) in cell-free culture supernatant were measured using ELISA kits with a microplate reader equipped with GEN5 software.

Citations: Publications based on the dataset

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Authors

Chol Seung Lim (oxr9@cdc.gov)
Ja Kook Gu (gum4@cdc.gov)
Qiang Ma (qam1@cdc.gov)

Contact

For further information, contact:
TMBB/HELD/NIOSH