MicroRNA-Mediated Krüppel-Like Factor 4 upregulation Induces Alternatively Activated Macrophage-Associated Markers and Chemokines Transcription in 4,4'-Methylene Diphenyl Diisocyanate Exposed Macrophages

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Introduction

Occupational exposure to 4,4'-methylene diphenyl diisocyanate (MDI), the most widely used monomeric diisocyanate (dNCO), is associated with occupational asthma (OA) development. Recruitment of immune cells to the lung microenvironment via secreted chemokines by alveolar macrophages may play a role during asthma pathogenesis. Our prior study identified that alternatively activated (M2) macrophage-associated markers and chemokines were induced by MDI/MDI-Glutathione (GSH)-mediated Krüppel-Like Factor 4 (KLF4) upregulation in macrophages and induced chemotaxis abilities to naïve T-cells and eosinophils; however, the underlying molecular mechanism(s) by which MDI upregulated KLF4 expression is unclear. Previously, we identified that two microRNAs (miRs) including *hsa-miR-206-3p* and *hsa-miR-381-3p* were significantly downregulated in MDI-GSH-exposed THP-1 macrophages. *In silico* analysis revealed that one *hsa-miR-206-3p* and *two hsa-miR-381-3p* predicted binding sites exist on the 3' untranslated region (UTR) of *KLF4* transcripts. We hypothesize that MDI/MDI-GSH exposure induces M2 macrophage-associated markers and chemokines through *hsa-miR-206-3p/hsa-miR-381-3p* mediated KLF4 upregulation in macrophages. The first aim of this study was to examine whether *hsa-miR-206-3p/hsa-miR-381-3p* regulates KLF4 expression in THP-1 macrophages through a posttranscriptional regulation mechanism. Our second aim was to determine whether *hsa-miR-206-3p/hsa-miR-381-3p* participates in KLF4-regulated M2 macrophage-associated markers and chemokines after MDI-exposure. After identifying the role of endogenous *hsa-miR-206-3p/hsa-miR-381-3p* in regulation of KLF4 transcription factor after MDI-exposure, we investigated the role of *hsa-miR-206-3p/hsa-miR-381-3p* in regulation of M2 macrophage-associated markers and chemokines' expressions in relation to the exposure to MDI.

Methods Collection

1. Cell culture and cell differentiation
    - THP-1 cells were obtained from ATCC.
    - Enhanced differentiated THP-1 macrophages were prepared using media containing 10 ng/ml phorbol 12-myristate 13-acetate (PMA) to induce differentiation for 3 days and then enhanced by refeeding fresh media after removing PMA containing media for additional 3 days.
2. MDI-GSH conjugation
    - 10 mM GSH solution was prepared in 200 mM sodium phosphate buffer (pH= 7.4).
    - MDI-GSH conjugation was prepared by adding MDI/acetone directly into GSH solution. End-to-end mixing for 1 h at 25 °C.
3. Transient transfection, KLF4 overexpression, and translational reporter assays
    - Plasmid DNAs were transfected into THP-1 macrophages using Mirus *Trans*IT-2020 transfection reagent according to manufacturer's instructions.

- miR-inhibitors or miR-mimics were transfected into THP-1 macrophages using Lipofectamine RNAiMAX transfection reagent according to manufacturer's instructions.
- Translational luciferase reporter assays were performed following transfection for 24 h The miR-inhibitors/-mimics were co-transfected with *KLF4*-3'UTR luciferase translational reporter plasmid, including the pRL-TK control into THP-1 macrophages for 24 h. The luciferase reporter activities were determined using Dual-Luciferase Reporter Assays according to manufacturer's instructions.

4. M2 macrophage-associated transcription factor, markers, and chemokines expression (transcripts and proteins)
    - Total RNA was isolated using *mirVana*™ miR isolation kit according to manufacturer's instructions.
    - TaqMan gene expression assays for *KLF4*, *CD206, TGM2, CCL17, CCL22* and *CCL24* were obtained from ThermoFisher Scientific.
    - Real-time PCR assays were performed on Applied Biosystems 7500 RT-PCR System.
    - Endogenous KLF4 protein were determined by western blot.
5. Validation of miR targets by Argonaute (Ago) immunoprecipitation
    - Immunoprecipitation (IP) of the miR-containing RNA inducing silencing complex (miR/RISC) and miR targeting mRNAs was performed using the miRNA target IP kit (Active Motif) according to manufacturer's instructions.
    - RNA was isolated from Immunoprecipitated miR/RISC/mRNA complexes using *mirVana*™ miR isolation kit according to manufacturer's instructions.
    - TaqMan gene expression assays for *KLF4*, *TGM2,* and *CCL22* were performed on RNA isolated from Immunoprecipitated miR/RISC/mRNA complexes.
    - Real-time PCR assays were performed on Applied Biosystems 7500 RT-PCR System.

Citations – Publications based on the dataset.

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