THE EFFECTS OF TNFα ON MIR-181A AND MIR-1 IN A549 LUNG CELLS

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Abstract: Inflammation is the underlying mechanism of many lung pathologies including lung fibrosis and cancer. TNFα, a pro-inflammatory cytokine released during acute lung injury (ALI) initiates a cascade of signaling pathways. Exposure to environmental pollutants leads to a considerable high incidence rate of ALI. MicroRNAs (miRs) are short strands of RNAs that regulate gene expression thus mediating signaling mechanisms in disease development. Studies have shown that miR-181a regulates inflammatory responses whereas miR-1 acts as a tumor suppressor. To further identify miRs mediated signaling in ALI, we used bioinformatics tools and identified collagen 3A1 (Col3A1) and Notch 2 novel potential targets for miR-181a, and thrombospondin-1 (TSP-1) for miR-1. Col3A1 and TSP-1 are components of extracellular matrix (ECM) and major players in fibrotic ECM in the lungs. Notch 2 is a member of the evolutionary conserved Notch family of receptors that regulate cell fate determination and differentiation during lung development and also plays a role in non-small cell lung cancer (NSCLC). In the present study, we investigated whether TNFα regulates miR-181a and miR-1 and identified targets Col3A1, Notch2, and TSP-1 in human A549 cells. Using A549, the regulation of miR-181a and miR-1 by TNFalpha and Col3A1, Notch 2, and TSP-1 were analyzed using real-time qPCR, western blot, and IHC. SnoU47 was used as endogenous control miR. A549 cells were exposed to TNFα (1 or 10 ng/ml) for 6 or 24 h. Total RNA was extracted using TRIzol method. MiR cDNA and cDNA were generated and analyzed by real-time qPCR with specific primers. Western blot and IHC were performed using specific antibodies and fluorescent microscopy. Low concentration of TNFα and short exposure (6 h) slightly decreased miR-181a (0.86- vs 1.0-fold change control). After 24 h, TNFα at low concentration inhibited miR-181a (0.27- vs 1.0-fold change). High concentration of TNFα and short exposure, increased miR-181a (1.86- vs 1.0-fold change). After 24 h, high dose of TNFα had no effect on miR-181a. Short exposure had no effect on Notch2 mRNA. After 24h, TNFα, regardless of the dose, increased Notch2 mRNA (1.7- and 2.3-fold change). TNFα suppressed TSP-1 mRNA. Ectopic miR-1 181a induced cell morphology changes whereas miR-1 had no effect. These results suggest that TNFα temporally and differentially regulates miR-181a and miR-1 and target genes, thus influencing inflammation-mediated signaling in lung injury. These data also suggest that miRs may represent a pharmacological target in lung injury.

Key words: ALI, TNFα, miR-181a, miR-1, COL3A1, Notch 2, TSP-1

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