Feasibility, distribution, and efficacy of an inhaled oligonucleotide mimic of miR-29 for pulmonary fibrosis induced by bleomycin in rats

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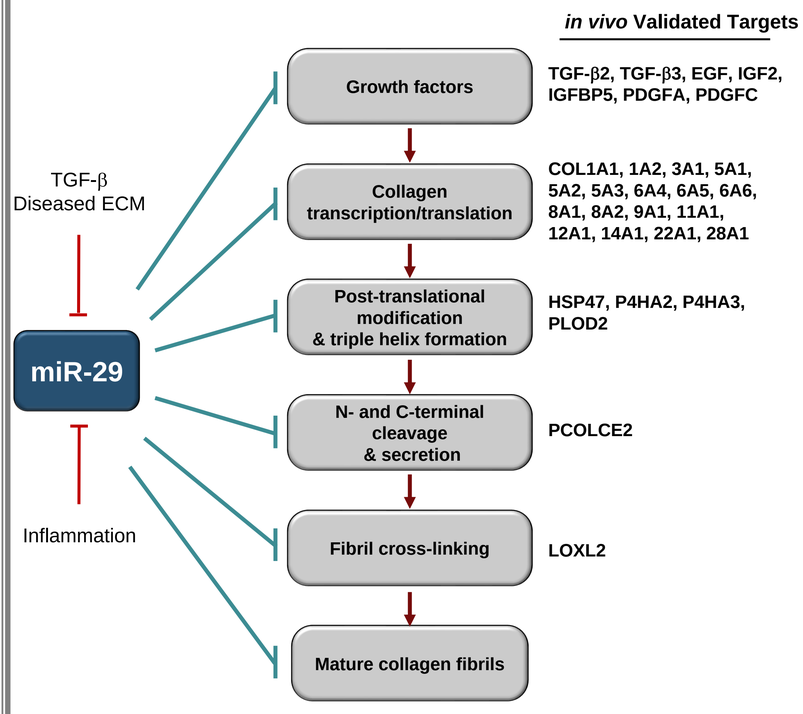
Abstract

miRNA-29 targets multiple profibrotic molecules and is down-regulated during fibrosis, including IPF. Previously, we showed a synthetic, chemically stabilized oligonucleotide mimic of miR-29b could repress collagen expression and abrogate pulmonary fibrosis in the bleomycin mouse model when administered I.V. Preliminary studies using a Scireq inExpose inhalation system yielded positive results with miR-29 mimic in bleomycin-treated mice. **Here, we sought to determine the feasibility of nebulizing miR-29 mimic (MRG-201) and to assess the distribution properties via nose-only inhalation in rats using a more sophisticated system.** miR-29 mimic aerosols were generated with an Aeroneb Solo nebulizer, analyzed by HPLC for compound integrity, and connected to a nose-only rat inhalation system. Rats were challenged with bleomycin at day 0 and received a single dose of miR-29 mimic at target doses of 0.5 or 5 mg/kg by nose-only inhalation and were sacrificed 6, 24, or 48 hours later for analysis. Using a dual probe hybridization assay, distribution to the lung was robust and dose-dependent, with significant clearance at 48 hours. Minimal compound was detected in the kidney (4.9 pmol/g) and plasma (46 pmol/mL) at 6 hours in the 5 mg/kg group only, with no detection observed in heart, liver, or spleen. We then looked at target regulation following multiple administrations at two different doses. Daily administration of 1.0 mg/kg demonstrated significantly better target regulation than 0.1 mg/kg. These data demonstrate the feasibility of nebulizing and delivering a mimic of miR-29 by nose-only inhalation, with robust and restricted delivery to the lung.

miR-29 Family: Antifibrotic miRNAs

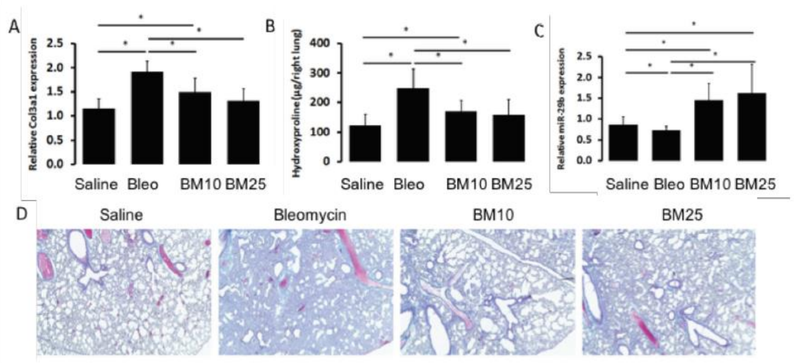
hsa, mmu, rno-miR-29a: 5'-UAGCACCAUCUGAAAUCGGUUA-3'
hsa, mmu, rno-miR-29b: 5'-UAGCACCAUUUGAAAUCAGUGUU-3'
hsa, mmu, rno-miR-29c: 5'-UAGCACCAUUUGAAAUCGGUUA-3'
Seed sequence



Methods

9-12 week old female mice received 10 or 25 mg/kg via aerosol (Scireq) 10 days after bleomycin treatment. We used 10 mice per group and the mice were sacrificed at day 21. Lung and BAL were harvested for histopathology and molecular analysis of fibrotic markers and drug delivery. For all experiments we used oligonucleotides synthesized at miRagen Therapeutics, Inc. For rat inhalation studies, all studies were performed at Lovelace Respiratory Research Institute. miR-29 mimic aerosols were generated with an Aeroneb Solo nebulizer, analyzed by HPLC for compound integrity, and connected to a nose-only rat inhalation system. Male rats were challenged with bleomycin at day 0 and received a single dose of miR-29 mimic at target doses of 0.5 or 5 mg/kg by nose-only inhalation and were sacrificed 6, 24, or 48 hours later for analysis. Plasma, lung, liver, kidney, spleen, and heart were collected for distribution analyses. For multi-day treatments, (n=5/group) rats were challenged at day 0 with bleomycin, and received 0.1 mg/kg/day or 1.0 mg/kg/day for 5 consecutive days starting at day 2. Rats were sacrificed at day 7, lung and plasma collected for target engagement and compound detection.

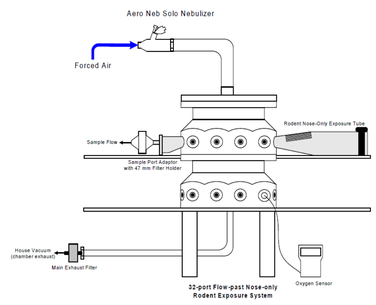
Scireq aerosol treatment of miR-29 mimic blunts bleomycin induced fibrosis in mice



Scireq Aerosol treatment with mir-29b 10 and 25mg/Kg. (A) Relative collagen 1 expression by qRT-PCR. (B) Hydroxyproline (C) Relative mir-29b levels. (D) Lung histology. BM10 – miR-29b 10mg/kg after bleomycin, BM25 – miR-29b 25 mg/kg after bleomycin. *p<0.05.

Rat aerosol exposure system with particle size assessment

A.



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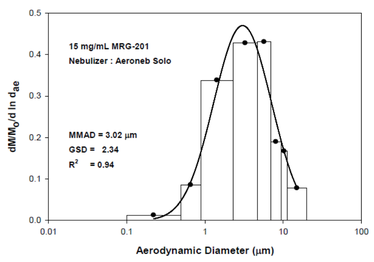


Test/Control Article	MMAD (µm)	GSD
Test Article (MRG-201)	3.02	2.34
Phosphate Buffer	3.46	2.91

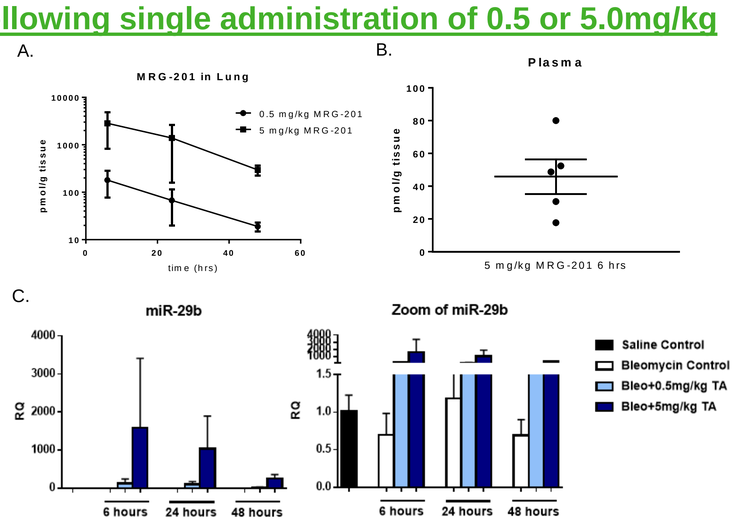
C.

Deposited Doses of MRG-201 from FY16-068 Inhalation Exposures.

Test Article Group Level	Total Avg. Aerosol Conc. (mg/L)	MRG-201 Avg. Aerosol Conc. (mg/L)	Exposure Time (minute)	Average Body Wt. (g)	Avg. Deposited Dose (mg/kg)
Low	0.787	0.318	16	163.5	0.40
High	0.826	0.334	160	172.9	4.21

Rat aerosol exposure system for miR-29 mimic (MRG-201) (A) Schematic of exposure system (B) Particle size distribution for miR-29 mimic aerosols and table for mass median aerodynamic diameter (MMAD). (C) Deposited doses for miR-29 mimic for the two test article groups. Targeted doses were 0.5 and 5.0 mg/kg.

miR-29 mimic levels in lung tissue and plasma following single administration of 0.5 or 5.0mg/kg

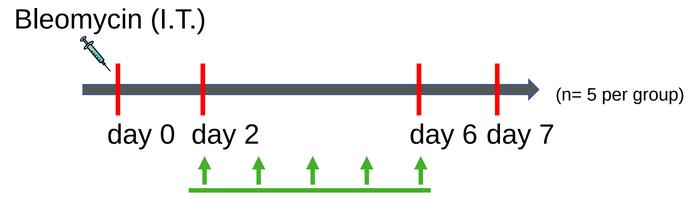


miR-29 mimic detectable in lung and plasma after inhalation (A) Detection of miR-29 mimic (MRG-201) in lung using a dual hybridization method. (B) Detection of miR-29 mimic (MRG-201) in plasma using the same method. (C) Detection of miR-29 levels by qRT-PCR showing relative abundance of mimic compared to endogenous miR-29 expression.

Biodistribution of miR-29 mimic

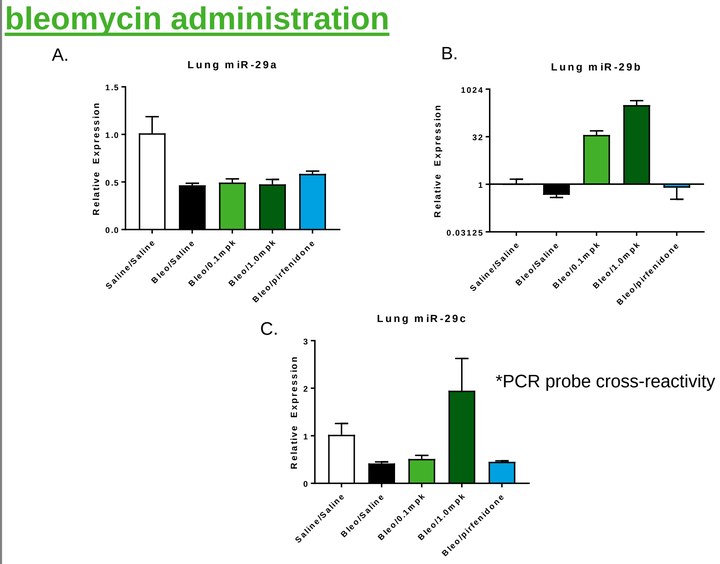
- Biodistribution analyses show robust and relatively dose-dependent exposure to the lung with significant clearance at 48 hours
- For heart, liver, and spleen, the average (or median) values were BLOQ.
- For kidney, there was very low levels detected at 6 hrs only for the 5 mg/kg, about 2x above the LLOQ (2.5 pmol/g; 42.7 ng/g).
- Plasma levels were only detectable at the 5 mg/kg dose 6 hours post dosing (46±24 pmol/mL); all other time points for both dose levels were BLOQ.

Design of multi-treatment study comparing two exposure levels in rat



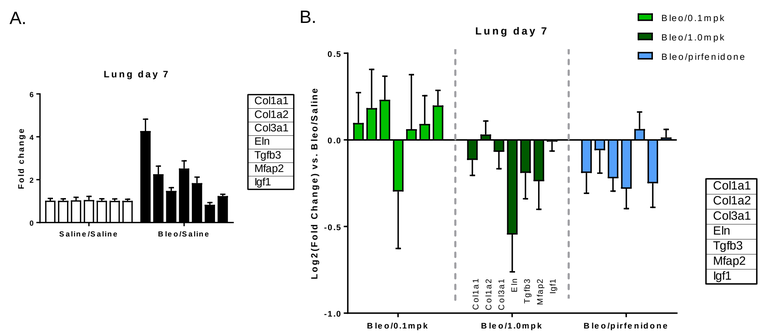
Daily treatment of:
- 0.1 mg/kg miR-29 mimic (inhalation)
- 1.0 mg/kg miR-29 mimic (inhalation)
- 100 mg/kg Pirfenidone (oral)

miR-29 family expression and detection after bleomycin administration



miR-29 family detection in lungs following bleomycin treatment in rats. (A) Relative miR-29a expression by qRT-PCR. (B) Relative detection of miR-29b by qRT-PCR, including endogenous expression and compound administered. (C) Relative miR-29c levels by qRT-PCR. Probe cross-reactivity makes it appear like miR-29c expression is increased. Note all miR-29 family members are down with bleomycin.

miR-29 targets are dose-dependently regulated by inhaled miR-29 mimics following bleomycin



Expression of miR-29 and fibrotic targets in lung after miR-29 mimic administration. Stress markers are increased with bleomycin at day 7 (A). miR-29 mimic and pirfenidone reduce expression of stress markers (B).

Conclusions

- miR-29 mimics can be nebulized and administered via nose-only inhalation to mice and rats.
- Distribution to tissues beyond the lung is very minimal, with only small detection in the kidney six hours after administration of the highest dose.
- Following multiple administrations, aerosolized miR-29 mimics at 1.0mg/kg can down-regulate target genes induced by bleomycin in rats.