Co-delivery of siPTPN13 and siNOX4 via (myo)fibroblast-targeting polymeric micelles for idiopathic pulmonary fibrosis therapy

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| Vendor       | Antibody                      | Catalog no. | Working dilution          |
|--------------|-------------------------------|-------------|---------------------------|
| Cell Signaling | Rabbit anti-PDGF Receptor α  | #3174       | WB, 1:1000 IF, 1:500 IHC, 1:500 |
| ABclonal     | Rabbit anti-PTPN13           | A13005      | WB, 1:1000 IF, 1:100 IHC, 1:100 |
|              | Rabbit anti-CD31             | A4900       | IF, 1:100                 |
| Abcam        | Rabbit Anti-α-SMA (APC)      | ab223921    | Flow Cyt, 1:200           |
|              | Rabbit anti-SP-C             | ab211326    | IF, 1:150                 |
| Santa cruz   | Mouse anti-AQP5              | sc-514022   | IF, 1:50                  |
|              | Mouse anti- PDGFRα           | sc-398206   | IF, 1:50                  |
| Bioss        | Rabbit anti-F4/80            | bs-11182R   | IF, 1:50                  |
| Boster       | Rabbit anti-NOX4             | BM4135      | WB, 1:1000 IF, 1:50 IHC, 1:50 |
|              | Rabbit anti-Collagen I       | BA0325      | WB, 1:1000                |
|              | Mouse anti-α-SMA             | BM0002      | WB, 1:1000 IF, 1:50       |
|              | Mouse anti-GAPDH             | BM3876      | WB, 1:5000                |
| Accession Number | Target gene | Forward Primer (5'→3') | Reverse Primer (5'→3') | Size (bp) | Tm (°C) |
|------------------|-------------|-------------------------|-------------------------|-----------|---------|
| NM_007392.3      | α-SMA       | CAGCCATCTTTTCATTGAGATGA | TGGTACCCCTCAGACAGAC     | 124       | 60      |
| NM_011204.2      | PTPN13      | CCTAAGGAAAATGGCCCTGG    | CCTCTTTGGCTCGAGAGG      | 140       | 60      |
| NM_001285833.1   | NOX4        | CCAATGTGTGGGATTTGTGT    | TCCTGCTAGGGACCTTCAGT    | 133       | 60      |
| NM_001289726.1   | GAPDH       | CCACTAGAGGATGCTGCC      | TACGGCCAAATCCGTACCA     | 124       | 60      |
Table S3. The characterization (size, PDI) of siPTPN13-loaded micelles with different polymers at various N/P<sub>feed</sub> ratio of 3, 6 and 10, respectively.

| Polymers       | N/P | Size from DLS in PBS (nm) | PDI (PBS) from DLS |
|----------------|-----|--------------------------|--------------------|
| PEI-g(15)-PEG-MAL | 3   | 78.8 ± 7.7               | 0.677              |
|                | 6   | 93.3 ± 8.3               | 0.563              |
|                | 10  | 88.4 ± 7.9               | 0.576              |
| PEI-g(20)-PEG-MAL | 3   | 29.8 ± 1.7               | 0.207              |
|                | 6   | 44.5 ± 2.9               | 0.137              |
|                | 10  | 68.8 ± 7.4               | 0.306              |
| PEI-g(24)-PEG-MAL | 3   | 128.5 ± 8.3              | 0.728              |
|                | 6   | 175.6 ± 7.8              | 0.672              |
|                | 10  | 145.7 ± 8.4              | 0.623              |
**Table S4.** The characterization (size, PDI, and Zeta potential) of siPTPN13-loaded micelles with various ratio of N/P feed. [DTSSP]/[NH2] = 0.4, n = 5.

| PIC micelles    | N/P | Mean size from TEM (n=50, nm) | Size from DLS in PBS (nm) | PDI (PBS) from DLS | Zeta Potential (mV) |
|-----------------|-----|------------------------------|---------------------------|--------------------|---------------------|
| Micelle-siPTPN13| 3   | 21.5 ± 0.77                  | 29.8 ± 1.7                | 0.207              | 2.6 ± 0.8           |
| Micelle-siPTPN13| 6   | 36.4 ± 2.8                   | 44.5 ± 2.9                | 0.137              | 7.5 ± 0.7           |
| Micelle-siPTPN13| 10  | 59.7 ± 5.5                   | 68.8 ± 7.4                | 0.306              | 13.6 ± 1.8          |
| Fab’ Micelle-siPTPN13 | 6   | 38.3±2.5                     | 45.9±3.2                  | 0.175              | 7.1±0.5             |
Table S5. The characterization (size, PDI, and Zeta potential) of siNOX4-loaded micelles with various ratio of N/P_{feed}. [DTSSP]/[NH₃] = 0.4, n = 5.

| PIC micelles        | N/P | Mean size from TEM (n=50, nm) | Size from DLS in PBS (nm) | PDI (PBS) from DLS | Zeta Potential (mV) |
|--------------------|-----|--------------------------------|---------------------------|-------------------|---------------------|
| Micelle-siNOX4     | 3   | 23.3 ± 0.9                      | 35.8 ± 2.2                | 0.365             | 3.3 ± 0.8           |
| Micelle-siNOX4     | 6   | 38.5 ± 1.9                      | 46.3 ± 3.2                | 0.145             | 6.9 ± 0.7           |
| Micelle-siNOX4     | 10  | 58.4 ± 3.2                      | 69.3 ± 5.4                | 0.344             | 13.4 ± 1.9          |
| Fab’ Micelle-siNOX4| 6   | 39.7 ± 3.9                      | 48.9 ± 2.7                | 0.165             | 6.1 ± 0.6           |
Table S6. The size and PDI of siRNA-micelles (N/P<sub>feed</sub> = 6) with various ratio of [DTSSP]/[NH<sub>3</sub>] as measured with DLS, n = 5.

| Ratio of [DTSSP]/[NH<sub>3</sub>] | 0     | 0.2   | 0.4   | 0.6    | 0.8    |
|----------------------------------|-------|-------|-------|--------|--------|
| Micelle-siPTPN13 (Size, nm)     | 41.3 ±1.9 | 42.7±1.7 | 44.5±2.9 | 93.3±5.8 | 123.5±8.3 |
| Micelle-siPTPN13 (PDI)          | 0.137 | 0.142 | 0.137 | 0.388 | 0.476 |
| Micelle-siNOX4 (Size, nm)       | 46.4±1.4 | 47.8±2.6 | 46.3±3.2 | 97.5±4.8 | 138.4±9.2 |
| Micelle-siNOX4 (PDI)            | 0.126 | 0.152 | 0.145 | 0.402 | 0.488 |
Table S7. The characterization of siRNA-loaded micelles, the ratio of N/P is 6 and [DTSSP]/[NH2] is 0.4 for all samples (n = 5).

| PIC micelles                  | Mean size from TEM (n=30, nm) | Size from DLS in PBS (nm) | PDI (PBS) from DLS | Zeta Potential (mV) |
|-------------------------------|-------------------------------|---------------------------|--------------------|---------------------|
| Micelle-siPTPN13              | 36.4±2.8                      | 44.5±2.9                  | 0.137              | 7.5±0.7             |
| Micelle-siNOX4                | 38.5±1.9                      | 46.3±3.2                  | 0.145              | 6.9±0.7             |
| Micelle-(siPTPN13+siNOX4)     | 37.3±2.6                      | 45.7±1.9                  | 0.137              | 9.3±0.6             |
| Fab’ Micelle-siPTPN13         | 38.3±2.5                      | 45.9±3.2                  | 0.175              | 7.1±0.5             |
| Fab’ Micelle-siNOX4           | 39.7±3.9                      | 48.9±2.7                  | 0.165              | 6.1±0.6             |
| Fab’ Micelle-(siPTPN13+siNOX4)| 41.5±2.9                      | 48.2±2.6                  | 0.177              | 8.4±0.8             |
| Fab’ Micelle-siControl        | 42.7±3.3                      | 49.4±3.1                  | 0.183              | 8.1±0.3             |
Table S8. The hydrodynamic diameter (DH) of Fab’ siRNA-loaded micelles as measured by FCS. The ratio of N/P is 6 and [DTSSP]/[NH₂] is 0.4 for all samples (n = 5).

| PIC micelles | DH in HEPES (nm) | DH in 10% serum included HEPES (nm) |
|--------------|-----------------|-----------------------------------|
| Fab’ Micelle-siPTPN13 | 48.3±3.1 | 48.9±2.7 |
| Fab’ Micelle-siNOX4 | 46.9±2.8 | 45.5±2.1 |
| Fab’ Micelle-(siPTPN13+ siNOX4) | 51.1±2.7 | 51.4±2.3 |
Figure S1. The $^1$H-NMR spectra of PEI-g(20)-PEG-MAL in D$_2$O at 25°C. a: the protons from maleimide (-CH=CH-); b: protons from PEG (-CH2CH2O-); c: the protons from PEI (N-CH2CH2-n).
Figure S2. The crosslinking of siRNA-loaded micelles affects the mRNA levels in myofibroblasts. PTPN13 and NOX4 mRNA levels in myofibroblasts incubated with Fab’ micelles-(siPTPN13+siNOX4) which was crosslinked with various ratio of [DTSSP]/[NH₂], as measured by qRT-PCR. The ratio of N/Pfeed is 6:1. Data are shown as the means ± SD.
Figure S3. Mice (n = 10 in each group) received either saline or bleomycin (BLM, 5 mg/kg body weight) intratracheally. Mice were sacrificed 21 days later. The colocalization of PDGFRα with AQP5 (AT I cell marker), SP-C (AT II cell marker), CD31 (endothelial cell marker) or F4/80 (macrophage marker) was determined by immunofluorescence assay.
**Figure S4:** The percentage of micelles-positive cells of Fab’ micelle-siRNA treatment group gradually decreased with the increase of concentration of PDGFRα antibody. Right panels: Quantified data of micelle⁺ cells. Results are expressed as means ± SD (n = 5; *p < 0.05, **p < 0.01).
**Figure S5:** Fab’ conjugated dual siRNA (siPTPN13 and siNOX4)-loaded micelles successfully reduced the expression of both PTPN13 and NOX4 *in vitro*. The mRNA levels of PTPN13 and NOX4 in (myo)fibroblasts with the administration of control siRNA, siRNA-loaded micelles with and without conjugation of Fab’, respectively, were examined by qRT-PCR. The siRNA concentration was 5 μg/mL for all the samples of control siRNA, siRNA-loaded micelles with and without conjugation of Fab’. Data are shown as means ± SD (*p < 0.05).
Figure S6. Biosafety evaluation of Fab’-dual siRNA (PTPN13 and NOX4)-loaded micelles in vivo. Histopathologic analyses of H&E-stained tissue sections of main organs (heart, liver, spleen, lung, and kidney) of mice after the indicated treatment.