Epithelial cell death is an important contributor to oxidant-mediated acute lung injury

SUPPORTING INFORMATION

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METHODS

Immunostaining. AT2 cells were plated on the coverslip and infected with 20PFU/cell of Ad-SOD2 for 48 hours. Prior to staining cells were washed in PBS three times and fixed in 3.7% formaldehyde in PBS for 5 minutes at room temperature (RT). After fixation cells were washed three times in PBS and then permeabilized in 90% methanol in PBS for 5 minutes at RT. Cells were then washed in PBS and incubated 30 min at RT in blocking buffer 3% FBS in PBS. The primary antibody for Calnexin (BD Biosciences, cat#610523)was diluted in blocking buffer 1:300 and incubated at RT for 1h followed by three washes in PBS. Primary antibody for SOD2 (Assay Designs, cat#SOD-111) was diluted in blocking buffer 1:150 and incubated at RT for 1h followed by three washes in PBS. Secondary antibodies, goat anti mouse(488) and chicken anti rabbit (594)both from Molecular Probes were diluted 1:200 in blocking buffer and incubated for 30 minutes at 37C. Cells were then washed in PBS and mounted on the microscope cover glass. Imaging was performed on the Zeiss UV LSM510 META microscope.

Fluorescent imaging of mito-Ro-GFP. Cells grown and treated on glass coverslips were mounted on an environmentally controlled stage of an epifluorescent microscope (controlled temperature) and perfused with hyperoxic (95% O_2, 5% CO_2) or normoxic (21% O_2, 5% CO_2) media (media bubbled with gases generated using a gas mixing flow meter) on the stage of an epifluorescent microscope equipped with filters to measure the ratio of emission (535 nm) after excitation at 410 and 490 nm) as previously described (1).

Staining for β-galactosidase activity. The lungs were fixed by the instillation of 2% formaldehyde and 0.2% glutaraldehyde in PBS (15 minutes 4°C) prior to the intratracheal

administration of a mixture of 5 mM $K_4Fe(CN)_6$-$3H_2O$, 5 mM $K_3Fe(CN)_6$, and 2 mM $MgCl_2$ in PBS with 0.5 mg/ml of X-gal solution (Sigma Aldrich, St. Louis, MO). The reaction was allowed to proceed overnight at 37°C prior to a final wash with PBS. For frozen lung sections, the lungs were perfused with OCT then snap frozen. Ten micron sections were then thawed, fixed and stained as described above (2).

FIGURE LEGENDS

Figure S1. A human lung adenocarcinoma cell line (A549) was infected with an adenovirus encoding mito-Ro-GFP and 48 hours later was stained with MitoTracker Red and mounted for imaging using confocal microscopy (A). Identically infected cells were mounted on an environmentally controlled stage of an epifluorescent microscope and perfused with hyperoxic (95% O_2, 5% CO_2) or normoxic (21% O_2, 5% CO_2) media. Oxidation of the probe was repeatedly measured in multiple cell regions over 60 minutes (B). Primary rat alveolar type II cells were fixed and immunostained using an antibody against SOD2 (left) or calnexin (right). Distinct staining patterns were observed (C).

Figure S2. Rosa 26R LacZ reporter mice on a C57BL/6 background were intratracheally treated with Ad-Null (A,B) or Ad-Cre (C,D) (1 X 10^9 pfu/animal) in 50% surfactant vehicle balance TE and 7 days later the lungs were harvested and stained for β-galactosidase activity through a tracheostomy tube (A and C). In another set of identically treated animals, the lungs were snap frozen with OCT, sectioned (10 μm) and stained for β-galactosidase activity. (A and C) are representative photographs of intact lacZ stained lungs shown from an anterior (left) or inferior (right) view (~1X). (B and D) are representative photomicrographs of lacZ stained frozen lung sections obtained using a phase contrast (left) or brightfield (right) filter (100X).

REFERENCES

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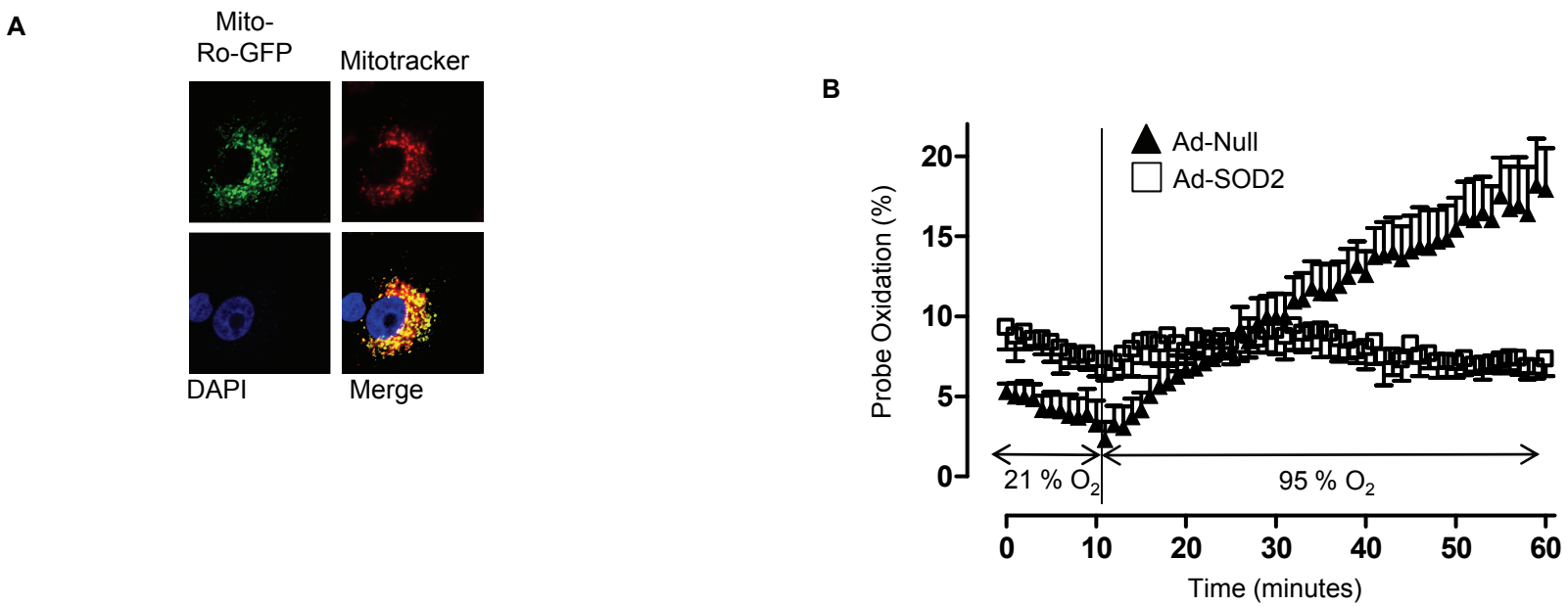
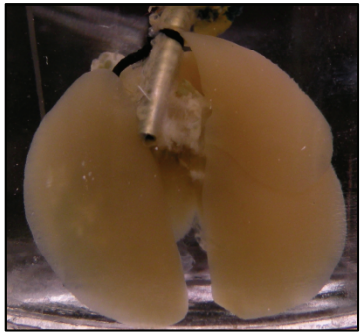

A
Mito-
Ro-GFP
Mitotracker
DAPI
Merge
B
Ad-Null
Ad-SOD2
Probe Oxidation (%)
20
15
10
5
0
21 % O2
95 % O2
0
10
20
30
40
50
60
Time (minutes)
C
SOD2
Calnexin

Figure S1

Ad-Null

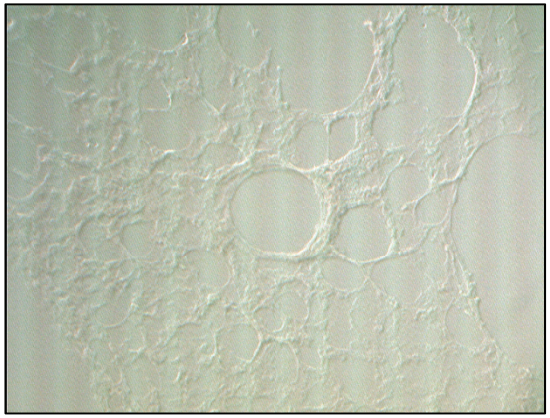
A

Anterior



Inferior

B

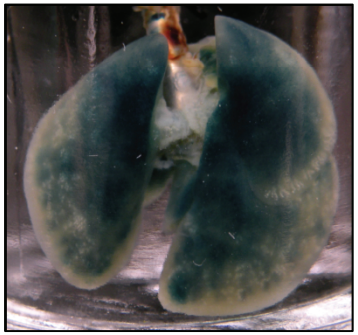


Phase

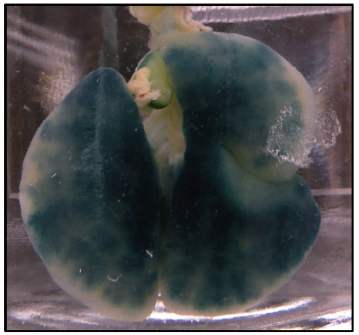
Brightfield

Ad-Cre

C

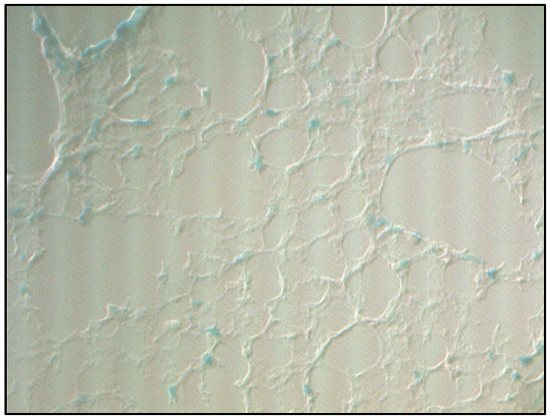


Anterior

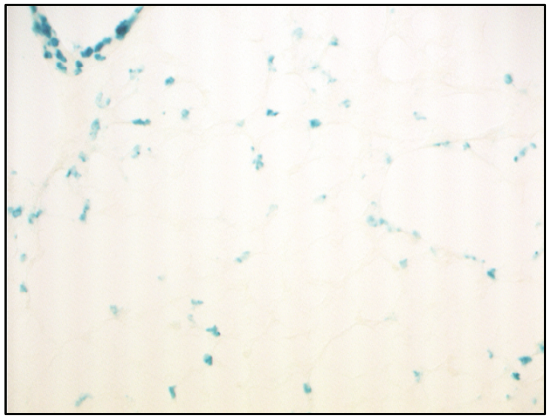


Inferior

D



Phase



Brightfield

Figure S2