Imbalance between cysteine proteases and inhibitors in a baboon model of bronchopulmonary dysplasia*

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ONLINE DATA SUPPLEMENT

Methods

Antibodies

Cathepsin B antibody was purchased from Athens Research and Technology, Inc (Athens, GA). Cystatin C and CD68 antibodies were purchased from Dakocytomation (Carpinteria, CA). Pro-surfactant protein C (Pro-SPC) antibody was purchased from Chemicon International (Temecula, CA). Cathepsin L (mAb 33/1), cat K, cat S, and cat H antibodies were generated and characterized as previously described (*1-6*). Cystatin B antibody, clone 4A10 was raised and characterized as previously described (*7*) (Figure E1). Mouse and rabbit primary antibody isotype controls were purchased from Zymed Laboratories (South San Francisco, CA). For immunoblotting, the primary antibodies were used at the following dilutions: anti- cat B, 1:3000; anti-cat H, 1:1000; anti-cat K, 1:1000; anti-cat L, 1:2500; anti-cat S, 1:1000; cystatin B, 1:2000; cystatin C, 1:1000. For immunohistochemistry, the primary antibodies were used at the following dilutions: anti-cat B, 1:800; anti-cat H, 1:1000; anti-cat L, 1:1000; anti-cat S at 1:400; anti-cystatin B, 1:200; anti-CD68, 1:100; anti-SPC, 1:1000.

References

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Hum Genet.

Figure Legend for online supplement

Figure E1. Specificity of monoclonal anti-cystatin B antibody, clone 4A10. Monoclonal antibodies against cystatin B were raised as previously described (34). Epitope mapping revealed that MAb, clone 4A10 recognized the same epitope (-ENKKFPVFK-) as the previously characterized MAb, clone 2E7 (34). To determine if 4A10 cross-reacts with cystatin A, recombinant cystatin A and B proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and detected by using the anti-cystatin B Mabs, clones 2E7, 4A10, 4C1, IA1, or an anti-cystatin A MAb.

Figure E2. Pro-SPC and cat H immunoreactivity on serial sections from a BPD group baboon lung sample. Arrows indicate type 2 alveolar epithelial cells. Scale bar, 100 μm.

Table E1. Oligonucleotide primers used in quantitative PCR reactions

Gene	Forward primer	Reverse Primer
Cathepsin B	5'-TGTGTATTCGGACTTCCTGCT-3'	5'-GTGTGCCATTCTCCACTCC-3'
Cathepsin H	5'-CATTTGCCAGCAACTGGAGGAA-3'	5'-ATTGGTTCAGTGCCATTTTAAATG-3'
Cathepsin K	5'-TGAGGCTTCTCTTGGTGTCCATAC-3'	5'-AAAGGGTGTCATTACTGCGGG-3'
Cathepsin L	5'-ACCAAGTGGAAGGCGATG-3'	5'-TCCCTTCCCTGTATTCCTG-3'
Cathepsin S	5'-ACTCAGAATGTGAATCATGGTG-3'	5'-TTCTTGCCATCCGAATATATCC-3'
Cystatin B	5'-GTTTAAGGCCGTGTCATTCA-3'	5'-GCTTTGTTGGTCTGGTAGTT-3'
Cystatin C	5'-TCTTTCCAGATCTACGCTGTGC-3'	5'-TGGGAGGTGTGCATAAGAGGT
CD 68	5'-CAACAAGCAATAGCACTGCCACCA-3'	5'-TGTTGGATGAACCGTGGCATTTCC-3'
GAPDH	5'-GGTGGTCTCCTCTGACTTC-3'	5'- CTCTTCCTCTTGTGCTCTTG-3'

Figure E1

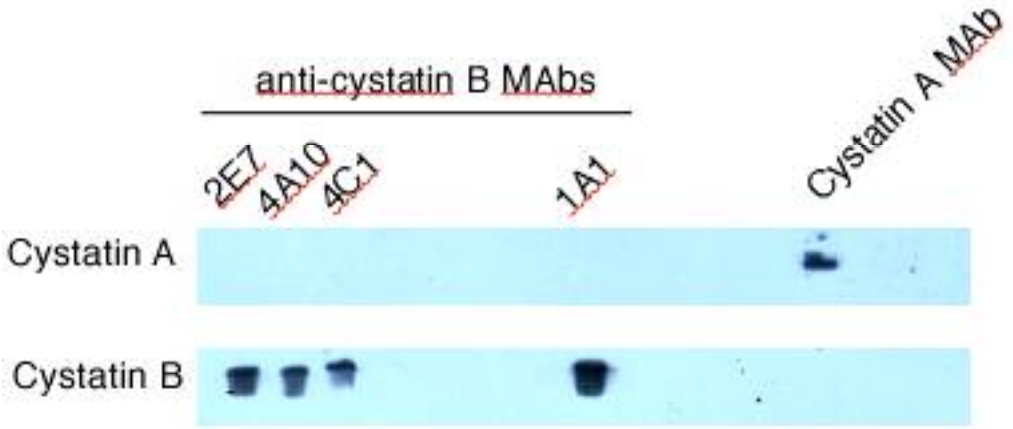
anti-cystatin B MAbs
2E7
4A10
4C1
1A1
Cystatin A MAb
Cystatin A
Cystatin B

Figure E2