

Respiratory MUC1 Mucin Interacts with Calcium-Modulating Cyclophilin Ligand (CAML)

Erik P. Lillehoj¹, Wei Guang¹, and K. Chul Kim²

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Abstract

Rationale: MUC1 is a type I transmembrane mucin glycoprotein expressed on the surface of respiratory epithelial cells. Its cytoplasmic C-terminus (CT) is evolutionarily conserved and mediates intracellular signal transduction (1). MUC1 expressed by breast cancer cell lines or transfected HEK293T cells initiates a Ca²⁺-based signaling response (2), but the molecular mechanism remains to be determined.

Methods: To better understand MUC1-dependent signaling, we searched for proteins binding to its CT using a yeast two-hybrid system with MUC1 as bait and a human airway epithelial cell cDNA library as prey. To confirm the results of the yeast two-hybrid interaction, expression constructs of the cMyc-tagged MUC1 CT and HA-tagged CAML were transiently transfected into HEK293T cells for coimmunoprecipitation (coIP), cross-linking, and confocal immunofluorescence microscopy.

Results: Of the positive clones encoding proteins that interacted with the MUC1 CT, the majority coded for CAML, a protein involved in Ca²⁺ signaling. The MUC1 CT interacted with CAML as revealed by growth on selective media and *in situ* X-α-galactosidase activity of yeast double transformants or progeny from matings of single transformants. Binding of the MUC1 CT to CAML was confirmed by reciprocal coIPs (MUC1 CT IP + CAML IB; CAML IP + MUC1 CT IB). By deletion mutagenesis, only the MUC1 CT and not its extracellular tandem repeat region or SEA domain interacted with CAML. Likewise, the region of CAML binding to MUC1 was localized to its N-terminus. Protein cross-linking and confocal microscopy also verified the MUC1-CAML interaction.

Conclusion: Our results identified CAML as a novel binding partner of the MUC1 CT and suggest that MUC1 plays a role in Ca²⁺ signaling.

References

1. Lillehoj EP, Kim KC (2003) Arch Pharmacol Res 25:770.
2. Rahn JJ et al. (2004) J Biol Chem 279:29386.

Acknowledgments

NIH grants ES013483 and AI072291, and the Cystic Fibrosis Foundation.

Results

Figure 1. MUC1 and CAML Plasmid Constructs

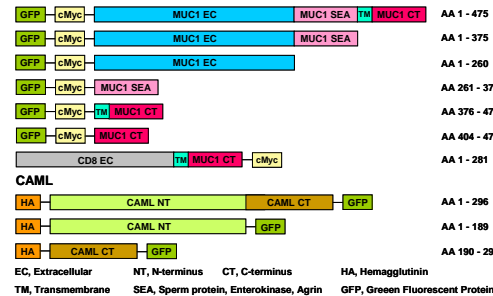
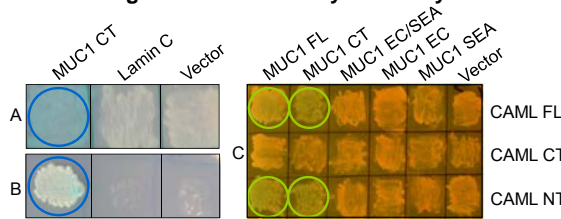


Figure 2. Sequence of Isolated CAML cDNA

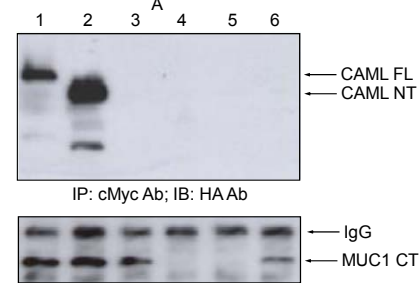


Figure 3. Yeast Two-Hybrid Analysis

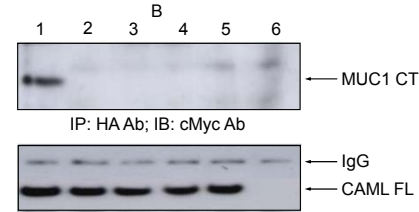


(A) Yeast double transformants containing CAML-pGADT7 plus MUC1 CT, Lamin C (irrelevant insert), or pGBKT7 empty vector on low stringency SD/-Leu/-Trp medium and reacted with X-α-Gal. Positive interactions are indicated by blue colonies (circled). (B) Transformants on high stringency SD/-Ade/-His/-Leu/-Trp medium with X-α-Gal. Positive interactions are indicated by colony growth. (C) The indicated MUC1 constructs in pGBKT7 and yeast strain Y187 were mated with the indicated CAML constructs in pGADT7 and yeast strain AH109, and plated on low stringency medium with X-α-Gal. Positive interactions are indicated by colored colonies (circled). FL, full length.

Figure 4. MUC1-CAML CoIP From Cell Lysates



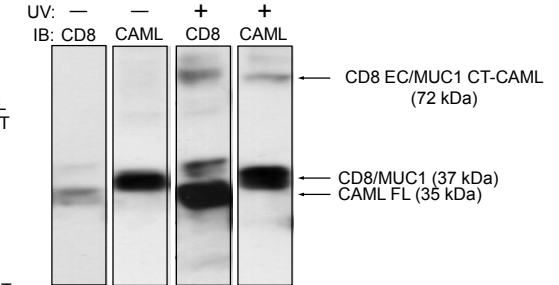
- 1, cMyc-MUC1 CT + HA-CAML FL
- 2, cMyc-MUC1 CT + HA-CAML NT
- 3, cMyc-MUC1 CT + HA-CAML CT
- 4, GFP only
- 5, pcDNA empty vector
- 6, cMyc-MUC1 CT only



- 1, HA-CAML FL + cMyc-MUC1 CT
- 2, HA-CAML FL + cMyc-MUC1 EC/SEA
- 3, HA-CAML FL + cMyc-MUC1 EC
- 4, HA-CAML FL + cMyc-MUC1 SEA
- 5, HA-CAML FL only
- 6, pcDNA empty vector

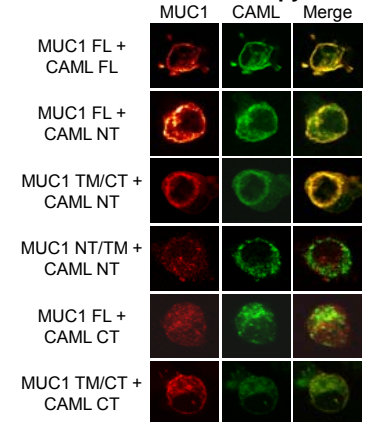
HEK293T cells were cotransfected with plasmids encoding the indicated cMyc-MUC1 or HA-CAML constructs, or pcDNA empty vector, and cell lysates were subjected to reciprocal coIP analysis with anti-cMyc and anti-HA antibodies (Ab). (A) IP: cMyc Ab (MUC1); IB: HA Ab (CAML). (B) IP: HA Ab (CAML); IB: cMyc Ab (MUC1).

Figure 5. MUC1-CAML Intracellular Cross-linking



HEK293T cells were cotransfected with plasmids encoding CD8 EC/MUC1 CT or CAML FL, the cells were metabolically labeled with photomethionine and photoleucine (Pierce), exposed or nonexposed to UV (365 nm) for 14 min, and cell lysates were analyzed by immunoblotting with CD8 or CAML antibodies. Note the appearance of the cross-linked CD8/MUC1-CAML complex after UV exposure.

Figure 6. MUC1-CAML Colocalization by Confocal Microscopy



HEK293T cells were cotransfected with plasmids encoding the indicated MUC1 and CAML constructs, the cells were fixed on glass slides, stained with MUC1 (red) and CAML (green) antibodies, and examined using a Zeiss LSM410 confocal microscope. Note colocalization of MUC1 FL or MUC1 CT with CAML FL or CAML NT after merge (yellow).



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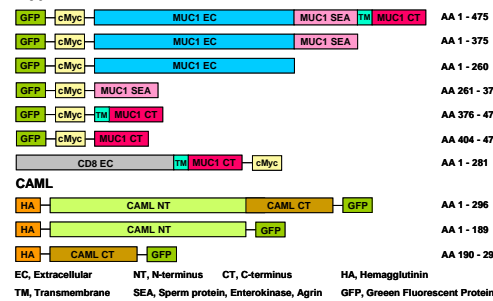
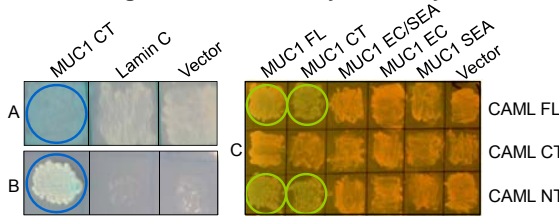


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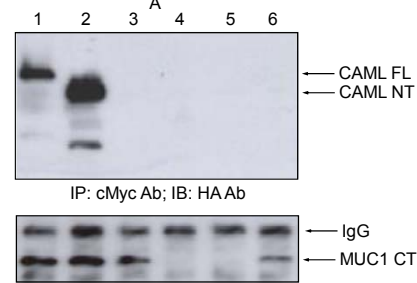


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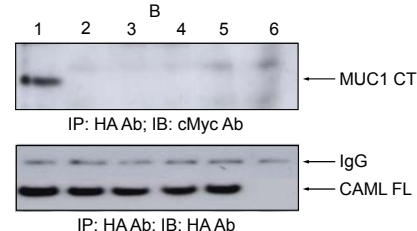


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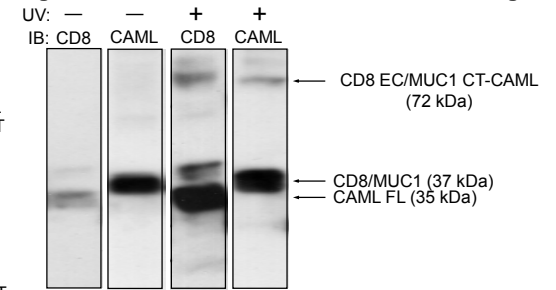
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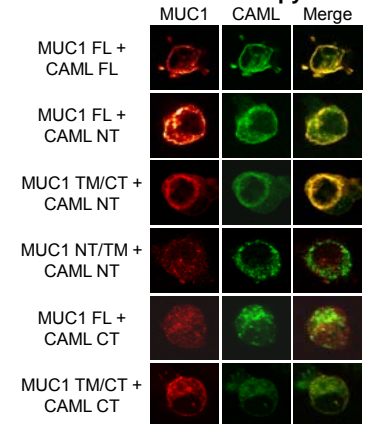
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