Involvement of PI3K, Src, and Grb2 in MUC1 Signaling

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ABTRACT

Background. MUC1 is expressed by airway epithelial cells and contains 7 Tyr residues in its cytoplasmic (CT) domain as potential signaling phosphorylation sites. Four Tyr residues are located within sequences that, when phosphorylated, constitute consensus motifs for signaling pathways: 1) Y^{20}HPM (PI3K), 2) Y^{35}VPP (PLC-γ1), 3) Y^{46}EEV (Src), and 4) Y^{60}TNP (Grb2). MUC1 Tyr phosphorylation is constitutive and induced but the particular residue(s) have not been identified.

Methods. COS7 and HEK293 cells were transiently transfected with a cDNA encoding a recombinant protein containing the CD8 extracellular and MUC1 CT domains (CD8/MUC1). CD8/MUC1 Tyr phosphorylation was assessed by immunoprecipitation and immunoblotting before and after stimulation with CD8 mab. Tyr-to-Phe mutagenesis was used to identify the site(s) of phosphorylation.

Results. In COS7 cells, the MUC1 CT region in the CD8/MUC1 chimera was Tyr phosphorylated constitutively and following stimulation with CD8 mab. However, only stimulated Tyr phosphorylation was seen in transfected HEK293T cells. Combined mutagenesis of Y20, Y35,Y46, and Y60 completely blocked constitutive and induced phosphorylation in COS7 cells while a minor amount of induced Tyr phosphorylation remained in HEK293T cells. Analysis of single and double Tyr-to-Phe mutants suggested Y20, Y35, and Y60 were phosphorylated following CD8 mab stimulation of both cells. 2D gel electrophoresis suggested multiple (3-4) Tyr phosphorylations in the CD8/MUC1 chimera following CD8 mab stimulation supporting the mutagenesis results.

Conclusions. These results suggest that PI3K, Src, and Grb2 are involved in the signaling function of MUC1.

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INTRODUCTION

Mucins comprise a family of secreted and membrane-bound glycoproteins encoded by 13 *MUC* genes. MUC1 is the best characterized membrane mucin, composed of an NH_2-terminal extracellular (EC) domain with a variable number of O-glycosylated tandem repeating peptide units, a transmembrane (TM) domain, and a cytoplasmic tail (CT) domain. Nearly 1/3 of the 72 amino acid CT domain consists of Tyr, Ser, and Thr residues that may serve as phosphorylation sites for intracellular signaling. Of the 7 Tyr residues, 4 are located within sequences that, when phosphorylated, constitute consensus motifs for signaling pathways: 1) Y^{20}HPM (phosphatidylinositol 3-kinase), 2) Y^{35}VPP (phospholipase C-γ1), 3) Y^{46}EEV (Src family kinases), and 4) Y^{60}TNP (Grb2). By analogy to other ligand-receptor signaling pathways, the presence of these Tyr residues suggests that ligand binding to the EC region of MUC1 initiates Tyr phosphorylation of its CT domain and subsequent activation of a signaling pathway. However, the identity of such ligand(s) and the site(s) of phosphorylation are unknown.

To further investigate Tyr phosphorylation of the MUC1 CT region, we constructed and characterized a recombinant protein containing the EC and TM regions of CD8 linked to the MUC1 CT region (Meerzaman et al., 2000). CD8 mab treatment of COS7 cells transfected with CD8/MUC1 increased Tyr phosphorylation and activated a Ras-MEK-ERK signaling pathway (Meerzaman et al., 2001). In this report, we performed Tyr-to-Phe site-directed mutagenesis to identify the sites of phosphorylation.

Meerzaman et al., AJP Lung 278:L625, 2000; Meerzaman et al., AJP Lung 281:L86, 2001

FIGURE 1
A CD8/MUC1 Chimera to Study MAP Kinase Signal Transduction

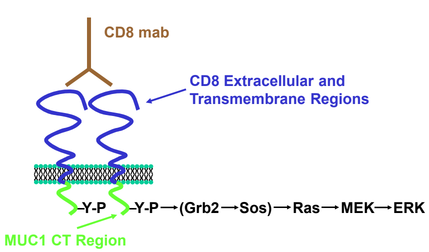


FIGURE 2
Construction and Characterization of CD8/MUC1 Expression Plasmid

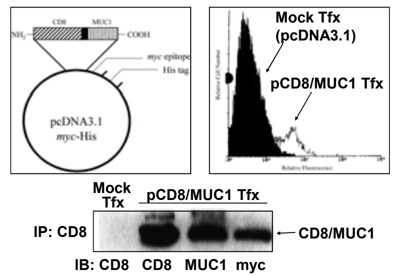


FIGURE 3
Tyrosine Phosphorylation of CD8/MUC1 in Transfected COS7 Cells

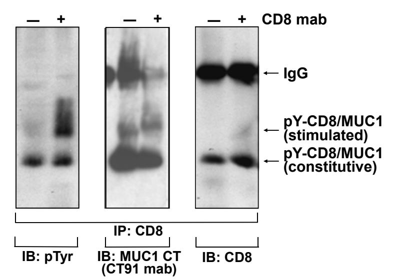


FIGURE 4
Tyrosine Phosphorylation of CD8/MUC1 in Transfected HEK293T Cells

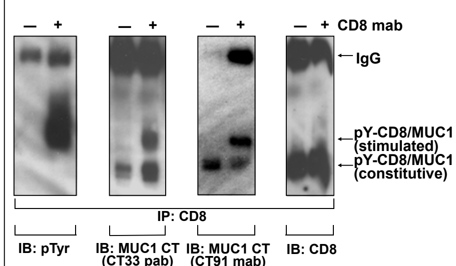


FIGURE 5
Phosphotyrosine Signaling Motifs in the MUC1 CT Region and Site-Directed Mutations

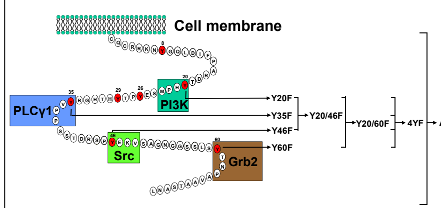


FIGURE 6
Tyrosine Phosphorylation of CD8/MUC1 Wild Type and ΔCT and 4YF Mutants in Transfected COS7 Cells

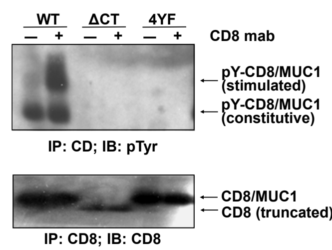
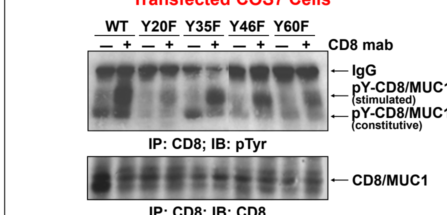


FIGURE 7
Tyrosine Phosphorylation of CD8/MUC1 Wild Type and Individual Tyr-to-Phe Mutants in Transfected COS7 Cells



Relative Band Intensities	
	Stimulated pY: Y20 < Y60 < Y46 < Y35 = WT
	Constitutive pY: Y20 = Y60 = Y46 < Y35 = WT

FIGURE 8
Tyrosine Phosphorylation of CD8/MUC1 Wild Type and Mutants in Transfected HEK293T Cells

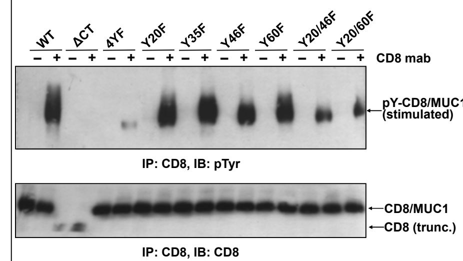
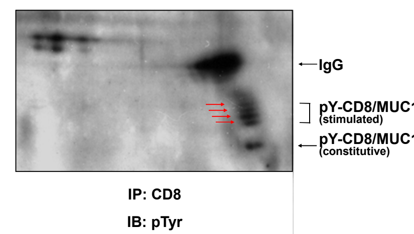


FIGURE 9
2D Gel Electrophoresis of CD8/MUC1 Expressed in COS7 Cells Indicates Multiple Tyrosine Phosphorylations



CONCLUSIONS

1. Tyr phosphorylation of the CD8/MUC1 chimera expressed in COS7 cells was constitutive and stimulated following treatment with CD8 mab. Stimulated Tyr phosphorylation, but not constitutive phosphorylation, was also seen in transfected HEK293T cells.
2. Deletion of the entire CT region (ΔCT mutant) blocked constitutive and stimulated CD8/MUC1 Tyr phosphorylation in COS7 cells and blocked stimulated phosphorylation in HEK293T cells.
3. Combined Tyr-to-Phe mutagenesis of the 4 CT region Tyr residues potentially involved in signaling (4YF mutant) blocked Tyr phosphorylation in COS7 cells while a minor amount remained in HEK293T cells.
4. Analysis of single and double Tyr-to-Phe MUC1 CT region mutants indicated Y20, Y46, and Y60 were phosphorylated following CD8 mab stimulation.
5. 2D gel electrophoresis suggested multiple (3-4) Tyr phosphorylations in the CD8/MUC1 chimera following CD8 mab treatment.