N-Glycosylation Regulates Chitinase 3-like-1 and IL-13 Ligand Binding to IL-13 Receptor α2

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ONLINE DATA SUPPLEMENT

ONLINE SUPPLEMENTAL DATA

MATERIALS AND METHODS

Yeast 2-hybrid assays

The full-length human IL-13Rα2 gene was amplified by PCR from human lung cDNA using the following primers (forward, 5'-AAG GTT TTC CAT ATG (Nde1) GGA GAA ATG GCT TTC GTT TGC-3'; reverse, 5'-GTC TCT TGA TAT CTC GAG (Xho1) TCT TCA TGT ATC ACG GAA-3'). IL-13Rα2 mutants that had defects in N-glycosylation were prepared from the wild type (WT) construct by replacing one or all of the N-glycosylation by site-directed mutagenesis (Table S2). The full length Chi3l1 and IL-13 genes were amplified as previously described by our laboratory (8, 21). The IL-13Rα2 DNA was cloned into the yeast two-hybrid BD vector at the *Nde1* and *Sal1* sites. The Matchmaker System 3 two-hybrid assay using *S. cerevisiae* (Clontech, Palo Alto, CA) was used to detect interactions between WT and mutated IL-13Rα2 and Chi3l1 or IL-13. The screening and identification of specific interacting molecules was undertaken according to the procedures described by our laboratory (8).

Cell culture

The THP-1 cells that were used were cytokine preconditioned as described previously (8, 23). MLE12 lung epithelial cell were obtained from the American Type culture Collection (ATCC; Rockville, MD).

Preparation and stimulation of peritoneal macrophages

Peritoneal macrophages were isolated from 7-week-old IL-13Rα2 null and WT mice according to the previously described procedures (19). which had been injected intraperitoneally 3 days earlier with 3ml of thioglycolate. The collected cells were washed twice with PBS and then cultured in DMEM containing 10%

heat-inactivated FBS, 1mM glutamine, 100 of IU/ml penicillin and 0.1 mg/ml of streptomycin at a density of $2x10^6$ cells/ml. The cells were then allowed to adhere for 3 h to a 24-well culture flask at 37°C in 5% CO2 and air. The cultures were ten washed twice with PBS to remove non-adherent cells before the addition of 1ml of fresh medium. Antibodies against F4/80 (565410, PharMingen, San Diego, CA) and FACS analysis on a FACSCaliber apparatus (Becton Dickinson, San Jose, CA) were used to assess cellular purity (>95%). In select experiments macrophages were incubated with rChi3l1 or vehicle control. Experiments with rChi3l1 were done in the absence of serum.

Co-Immunoprecipitation (Co-IP)

To assess interactions between IL-13Rα2 and its ligands, epithelial cells were co-transfected with IL-13Rα2 and plasmids (pcDNA3.1) containing either Chi3l1 or IL-13. Lysates from these cells were subjected to immunoprecipitation using anti-hIL-13Rα2 mouse monoclonal antibody (R&D systems, Minneapolis, MN) or antibodies against Chi3l1 (R&D systems) or IL-13 (Abcam, Cambridge, MA). Catch and Release v2.0 Reversible Immunoprecipitation System (EMD Millipore, Bedford, MA) was used for these Co-IP evaluations according as per the manufacturer's instruction. The precipitates were then evaluated by immunoblotting with antibodies against IL-13Rα2, Chi3l1 or IL-13 as previously described by our laboratory (8, 19).

Immunoblotting

Protein lysates were prepared from cultured cells or whole lungs using RIPA lysis buffers and subjected to immunoblotting using a modification of procedures described previously by our laboratory (5). ChemiDoc MP gel imaging system (BioRad) was used to capture the gel images and further image analysis including densitometry.

SUPPLEMENTAL TABLES

Table S1. Primer Sequences and Locations Used for Real time analysis of mRNA expression

Point mutation site	Primer Set Name	Primer Sequence
STT3A	STT3A-UP	TGTCGATGGCTGCTGTGTTAT
	STT3A-DN	CAGAAACCGGGTAGTCCGAT
STT3B	STT3B-UP	ATCCACGAGTTCGACCCGT
	STTB-DN	ACCATGCTCTTTCATCAAACCA
DAD1	DAD1-UP	TGAAGTTGCTGGACGCCTATC
	DAD1-DN	AAGCCAGAGAGGAACGAGTTG
N33	N33-UP	TTGCGCTACCTGCCTACTG
	N33-DN	CTGCTCCACTTTTTCAGCCAA
OST4	OST4-UP	TCGCCATCTTCGCCAACAT
	OST4-DN	TGCCACGTAGTGATAGAGGAC
RPN1	RPN1-UP	GCTTCATCCGTATCCGACTCA
	RPN1-DN	CCAGTTTCACTCGCATGGT
RPN2	RPN2=UP	CAGTGGTGTCCAATACATTCACG
	RPN2-DN	TGTTGAGCTGAGTCCAGTAGAT

Table S2. Primer Sequences and Locations Used to Generate hIL-13Rα2 Point Mutation.

Point mutation site	Primer Set Name	Primer Sequence
No Mutation (Full Length)	FL-AD1	AAG GTT TTC CAT ATG GGA GAA ATG GCT TTC GTT TGC
	FL-AD2	GTC TCT TGA TAT CTC GAG TCT TCA TGT ATC ACG GAA
115 AAT TO CAG	115-AD-FW	CAG GGA TCA GAA GTT CAA AGT TCC TGG GCA GAA ACT
	115-AD-RV	TGT GCA TTG CCA TGG TAA AAG CGT GTG TAT CTT CGC
168 AAT TO CAG	168-AD-FW	CAG TAC AAC TTG TTT TAC TGG TAT GAG CGG TTG GAT
	168-AD-RV	GGT ATC AAG AAG TAC ACC TAT GCC AGG TTT CCA AGA
215 AAT TO CAG	215-AD-FW	CAG GGA TCA TCA GAG AAC AAG CCT ATC AGA TCC AGT
	215-AD-RV	AAC ACA AAT ATA GAA ATC TTT ATA GTC TGA TGC CTC CAA
299 AAT TO CAG	299-AD-FW	CAG GAA ACC CGA CAA TTAT GCT TTG TAG TAA GAA GCA
	299-AD-RV	TGT TGT TTT CAA GGT GTA TGT TTC ATT TTC AAC TGT AGC

Supplemental Figure

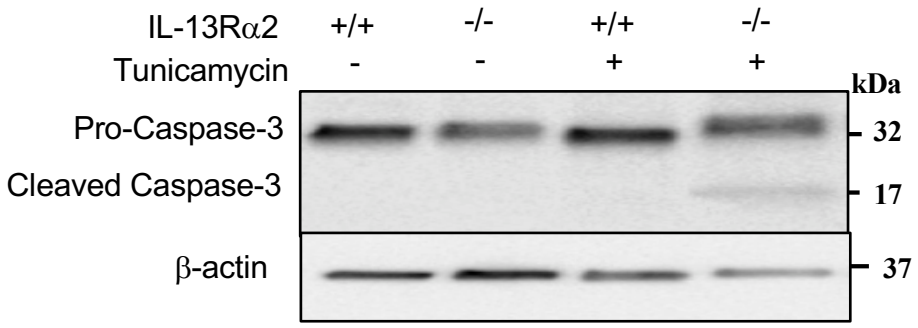

Fig. S1. Tunicamycin induced caspase 3 activation (cleavage) in the absence of IL-13Rα2. Peritoneal macrophages isolated from WT (+/+) and IL-13Rα2 null mutant mice (-/-) were stimulated with and without tunicamycin treatment (2mg/ml). After overnight incubation, cells were harvested and total cell lysates were subjected to immunoblot assays with anti-Caspase-3 antibody that detects both pro- and active (cleaved) forms of Caspase-3.