IL-13Rα2 uses TMEM219 in chitinase 3-like-1-induced signalling and effector responses

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Accessibility
Recent studies demonstrated that chitinase 3-like-1 (Chi3l1) binds to and signals via IL-13Rα2. However, the mechanism that IL-13Rα2 uses to mediate the effects of Chi3l1 has not been defined. Here, we demonstrate that the membrane protein, TMEM219, is a binding partner of IL-13Rα2 using yeast two-hybrid, co-immunoprecipitation, co-localization and bimolecular fluorescence complementation assays. Furthermore, fluorescence anisotropy nanodisc assays revealed a direct physical interaction between TMEM219 and IL-13Rα2-Chi3l1 complexes. Null mutations or siRNA silencing of TMEM219 or IL-13Rα2 similarly decreased Chi3l1-stimulated epithelial cell HB-EGF production and macrophage MAPK/Erk and PKB/Akt activation. Null mutations of TMEM219 or IL-13Rα2 also phenocopied one another as regards the ability of Chi3l1 to inhibit oxidant-induced apoptosis and lung injury, promote melanoma metastasis and stimulate TGF-β1. TMEM219 also contributed to the decoy function of IL-13Rα2. These studies demonstrate that TMEM219 plays a critical role in Chi3l1-induced IL-13Rα2 mediated signalling and tissue responses.
IL-13 Receptor α2 (IL-13Rα2) was originally described as a high affinity receptor for IL-13 that is distinct from the IL-13Rα1-IL-4Rα receptor heterodimer that IL-13 shares with IL-4 (refs 1,2). It was initially believed to be a decoy receptor for IL-13 because IL-13Rα2 only contains a 17 amino acid cytoplasmic tail that lacks a conserved box 1 region that has been shown to play a critical role in signal transduction3, and early studies highlighted its ability to diminish IL-13 responses1,4. However, more recent studies have challenged this ‘decoy’ concept by demonstrating that IL-13 also signals and regulates a variety of cellular and tissue responses via IL-13Rα2 (refs 2,6–12). Recent studies from our laboratory also demonstrated that IL-13 is not the sole ligand for IL-13Rα2 and that chitinase 3-like-1 (Chi3l1, also called YKL-40 in man and BRP-39 in the mouse) binds to, signals and regulates oxidant injury, apoptosis, pyroptosis, inflammasome activation, pathogen containment, and the induction of total and active TGF-β in oxidant-induced cell death and injury responses, lung fibrosis, melanoma metastasis and the induction of total and active TGF-β in oxidant-induced cell death and injury responses (Fig. 1e). In all cases this staining was appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e). In all cases this staining was appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e). In all cases this staining was appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e). In all cases this staining was appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e). In all cases this staining was appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e).

Results

TMEM219 binds and co-localizes with IL-13Rα2. To identify the binding partners of IL-13Rα2, Y2H screening was employed as previously described13. Multiple candidate molecules were identified when IL-13Rα2 was used as bait against a human lung cDNA library. Surprisingly, 10 of the 19 interacting clones that were obtained encoded TMEM219 (Fig. 1a). The interaction between IL-13Rα2 and TMEM219 was further validated using co-immunoprecipitation (Co-IP) and BiFC assays. In the former, 1HAEo lung epithelial cells were transfected with plasmids containing full-length complementary DNA encoding IL-13Rα2 or TMEM219, subjected to immunoprecipitation with antibodies against one moiety and the precipitate was analyzed using immunoblots (IBs) with antibodies against the other. In these experiments, the two moieties consistently travelled together with IP using antibodies against IL-13Rα2 precipitating TMEM219 and vice versa (Fig. 1b). Interestingly, TMEM219 did not interact in a similar manner with IL-13Rα1 (Fig. 1c). The BiFC assays further visualized this interaction in live cells. In these experiments 1HAEo cells were transfected with vectors that contain IL-13Rα2 or TMEM219 each of which contained a partial and complementing yellow protein (YFP) construct (Supplementary Fig.1a for detailed map of each construct). When these constructs co-localize, fluorescence can be appreciated. As shown in Fig. 1d, the fluorescence complementation was apparent after co-transfection of constructs of IL-13Rα2-V1 and TMEM219-V2 which no signal was noted in the cells transfected with individual constructs or cells transfected with individual constructs with negative control constructs (C–C chemokine receptor type 3 (C3CR)-V1 or -V2 or TMEM219-V1 or -V2; Fig. 1d and Supplementary Fig. 1b). Interestingly, when cells were incubated with rChi3l1 (500 ng ml⁻¹), a known ligand of IL-13Rα2 (ref. 13), the signal complementation after co-transfection of IL-13Rα2-V1 and TMEM219-V2 was significantly enhanced compared with non-treated cells (Fig. 1d; Supplementary Fig. 1b), suggesting that ligand binding to IL-13Rα2 significantly enhances the physical interaction between IL-13Rα2 and TMEM219. Similar results were seen when primary normal human bronchial epithelial cells (NHBE cells) were transfected with IL-13Rα2-V1 and TMEM219-V2 and treated with rChi3l1 (Supplementary Fig. 1c). In support of the notion that ligands of IL-13Rα2 augment IL-13Rα2-TMEM219 interaction, rIL-13 also increased this interaction in both HAEo transformed cells as well as NHBE cells (Supplementary Fig. 1b,c).

We next localized the expression of TMEM219 and IL-13Rα2 using confocal microscopy and double-label immunostaining. Lungs from IL-13 transgenic (Tg) mice were used in these evaluations because the expression of IL-13Rα2 and TMEM219 are significantly induced in these tissues. We noted that TMEM219 was widely expressed on the outer membranes and to a lesser degree in intracellular locations of the lung cells, with prominent expression in airway epithelial cells and macrophages (Fig. 1e). As we previously demonstrated13, IL-13Rα2 was readily appreciated both in an intracellular pool and on the outer membrane of macrophages (Fig. 1e). In all cases this staining was specific because it was not present in the absence of the primary antibody and it was successfully competed against with recombinant peptide. Importantly, TMEM219 and IL-13Rα2 colocalized on the outer cell membranes (Fig. 1e, arrows) but IL-13Rα2 had a more prominent intracellular pool. Lastly, the critical region of IL-13Rα2 that binds to full-length TMEM219 were determined using IL-13Rα2 constructs in the Y2H assay as described previously13. These studies demonstrated that the extracellular domain (ECD) of IL-13Rα2 plays a critical role in binding to TMEM219 (Fig. 1f; Supplementary Fig. 1d). When viewed in combination, these studies demonstrate that TMEM219 physically interacts with IL-13Rα2.

TMEM219 in nanodiscs binds IL-13Rα2-Chi3l1 complexes. We further characterized the interactions between TMEM219, IL-13Rα2 and Chi3l1 using nanodisc and fluorescence anisotropy (FA) (also known as fluorescence polarization) assays as described previously14. For these evaluations, we expressed
Chi3l1 and ectodomains of IL-13Rα2 (ecto-IL-13Rα2) using a baculovirus-insect cell expression system and TMEM219 proteins using a mammalian expression system. Purified TMEM219 protein was reconstituted in 13 nm nanodisc with an estimated stoichiometry of 1 copy per disc (Supplementary Fig. 2a,b). An empty nanodisc was used as a control for nonspecific binding. The changes in FA values obtained from these titrations were close or below the changes in FA of free labelled protein or labelled protein alone (Supplementary Fig. 2c). This demonstrates that the empty nanodisc did not bind to Chi3l1, or IL-13Rα2 complexed with Chi3l1. We next looked for interactions between the ecto-IL-13Rα2, Chi3l1 and the TMEM219 nanodisc. At the concentration range used in this experiment, we did not observe any direct interaction between TMEM219 nanodisc and Chi3l1 or IL-13Rα2 (Fig. 2a,b). However, TMEM219 nanodisc showed strong interactions with IL-13Rα2-Chi3l1 complexes with Kd values of approximately 288 nM (Fig. 2c). These data provided a direct assessment of IL-13Rα2 and Chi3l1 complexing with TMEM219 in vitro.

Roles of TMEM219 and IL-13Rα2 in HB-EGF production. Previous studies demonstrated that human 1HAEo cells produce HB-EGF via an IL-13Rα2-dependent mechanism. In accord with these findings, Chi3l1 also stimulated HB-EGF mRNA accumulation and protein elaboration (Fig. 3a,b). In both cases the silencing of IL-13Rα2 markedly

![Figure 1](image1.png)

**Figure 1 | TMEM219 physically interacts with IL-13Rα2.** (a) Y2H demonstration of the interaction between IL-13Rα2 and TMEM219. (b) 1HAEo cells were transfected with hIL-13Rα2 (IL-13Rα2) and/or human TMEM219 (TMEM219) expressing plasmids. Co-IP performed with either anti-IL-13Rα2 (1:500, AF146, R&D) or anti-TMEM219 (1:500, sc-244405, Santa Cruz) antibody, and the precipitates were evaluated using IB analysis as noted. (c) Direct interaction between TMEM219 and IL-13Rα1 was tested by Co-IP/IB assay using recombinant human (rh) TMEM219 (350 ng), IL-13 (300 ng, 213-ILB, R&D) and IL-13Rα1 (500 ng, 146-IR, R&D). His-tagged human TMEM219 was expressed and purified in HEK 293T cells using pcDNA 3.1 vector. Commercially available Recombinant human IL-13 and IL-13Rα1 (R&D systems) were used for this assay. (d) Interaction of IL-13Rα2 and TMEM219 on cell membrane was confirmed by BiFC assay. IL-13Rα2 and TMEM219 constructs were generated which contain fragments of the tagging protein Venus YFP fragments (V1 and V2). A similar approach was used to generate the negative control, CCR3 containing Venus YFP fragments. The indicated plasmids were transfected into 1HAEo cells, which were treated with rChi3l1 (500 ng ml⁻¹, 2599-CH, R&D), rIL-13 (20 ng ml⁻¹, 213-ILB, R&D) or vehicle control as noted. (e) Immunohistochemical demonstration of the co-localization of IL-13Rα2 and TMEM219 in lungs from IL-13 Tg mice. Fluorescence images were counterstained with 4’6-diamidino-2-phenylindole (DAPI) for nucleus identification. (f) Y2H characterization of the IL-13Rα2 sequences critical for binding to hTMEM219. The extracellular domain (ECD), transmembrane domain (TD), intracellular domain (ICD), signal peptide (SP) and sites of N-glycosylation (N) are illustrated. Fragment binding is illustrated with a + sign. (b,c) Representative of two separate western blot analysis. (d,e) Composite illustration taken from the noted four groups of mice (N>5) that behaved similarly.
diminished while the silencing of IL-13Rα2 did not alter these inductive events (Fig. 3a,b; Supplementary Fig. 3a,b). Chi3l1 also stimulated HB-EGF mRNA accumulation and protein production by primary mouse macrophages and this induction was also mediated via an IL-13Rα2-dependent mechanism (Fig. 3c; Supplementary Fig. 3c). To determine if TMEM219 played a role in these responses, we evaluated the ability of Chi3l1 to stimulate the expression and production of HB-EGF after siRNA silencing or genetic ablation of TMEM219. The siRNA silencing that was employed was target specific and decreased TMEM219, IL-13Rα2 or IL-13Rα1 expression by approximately >90% (Supplementary Fig. 4). Silencing of TMEM219 in 1HAEo cells and null mutations of TMEM219 in mouse peritoneal macrophages both significantly decreased rChi3l1-stimulated expression of HB-EGF (Fig. 3a–c; Supplementary Fig. 3a–c). Similar decreases in rChi3l1-stimulated HB-EGF expression were noted in the cells treated with monoclonal antibodies against TMEM219 compared with isotype controls (Fig. 3d). Importantly, TMEM219 silencing also caused a similar decrease in Chi3l1-induced HB-EGF production by NHBE cells. When viewed in combination, these studies demonstrate that Chi3l1 stimulates the production of HB-EGF by transformed and normal airway epithelial cells and macrophages via IL-13Rα2-dependent mechanisms and that TMEM219 plays a similarly essential role in these inductive cellular events.

**Figure 2 | Direct interaction of IL-13Rα2 complexes and TMEM219 nanodisc.** (a,b) Serial dilution of TMEM219 nanodisc (10–10,000 nM) were incubated with 10 nM of BIOPYD-labelled Chi3l1 or IL-13Rα2. No significant increase of fluorescence signals was noted in these nanodisc incubations. (c) Serial dilution of TMEM219 nanodisc (10–10,000 nM) were incubated with 10 nM of BIOPYD-labelled IL-13Rα2-Chi3l1 complexes. As the concentration of the nanodisc increases, changes in fluorescence signal increases until it reaches saturation. IL-13Rα2-Chi3l1 complexes directly bind to TMEM219 nanodisc with an affinity of 288 nM, respectively. All the panels are representative of a minimum of two separate experiments.

**Roles of TMEM219 and IL-13Rα2 in MAPK and Akt/PKB activation.** In previous studies, we demonstrated that Chi3l1 activates MAPK/Erk, Akt/PKB and Wnt/β-catenin signalling via IL-13Rα2-dependent pathways. To evaluate the roles of TMEM219 in these signalling responses, 1HAEo lung epithelial cells and murine peritoneal macrophages were stimulated with rChi3l1 with and without siRNA silencing or null mutations of TMEM219 (Fig. 4a–d). Chi3l1 activated MAPk/Erk and Akt/PKB in 1HAEo lung epithelial cells (Fig. 4a) and murine peritoneal macrophages (Fig. 4b). In both cell types, this activation was IL-13Rα2-dependent because it was significantly decreased by treatment with silencing siRNA or null mutations of IL-13Rα2 (Fig. 4a,b). In contrast, silencing IL-13Rα1 in 1HAEo cells did not alter the ability of Chi3l1 to activate these signalling pathways (Fig. 4c). Similar results were obtained when these cells were made deficient in TMEM219. Specifically, siRNA silencing or null mutations of TMEM219 significantly decreased Chi3l1-stimulated MAPk/Erk and Akt/PKB activation in these cells (Fig. 4a,b). Treatment of 1HAEo cells with neutralizing antibodies against TMEM219 also significantly reduced rChi3l1-stimulated MAPk/Erk and Akt/PKB activation when compared with isotype controls (Supplementary Fig. 5). Importantly, similar decreases in Chi3l1-induced MAPk/Erk and Akt/PKB signalling were seen when TMEM219 was silenced in primary NHBE cells (Supplementary Fig. 6). These studies demonstrate that TMEM219 and IL-13Rα2 play similar and critical roles in Chi3l1- stimulated activation of MAPK/Erk and Akt/PKB.

**Roles of TMEM219 and IL-13Rα2 in Wnt/β-catenin signalling.** We previously demonstrated that the intracellular 17 amino acids in IL-13Rα2 play a critical role in Wnt/β-catenin signalling but are not required for MAPK or Akt/PKB activation. In accord with these findings siRNA silencing or null mutations of IL-13Rα2 effectively blocked Chi3l1-induced β-catenin nuclear translocation in 1HAEo cells and murine peritoneal macrophages (Fig. 4d,e). In contrast, ablation of TMEM219 did not affect
Chi3l1-stimulated Wnt/β-catenin activation in 1HAEo cells (Fig. 4d) or macrophages (Fig. 4e). These studies demonstrate that, in contrast to the MAPK and Akt signalling pathways, Chi3l1 activates Wnt/β-catenin signalling via a TMEM219-independent mechanism.

Roles of TMEM219 and IL-13Rα2 in apoptosis and lung injury.

We recently demonstrated that Chi3l1 inhibits oxidant-induced cellular apoptosis and lung injury via an IL-13Rα2-dependent mechanism(s)13. Thus, studies were undertaken to define the role(s) of TMEM219 in these responses. In vitro treatment of 1HAEo cells with low dose H2O2 (200 μM) caused a modest increase in apoptotic cells expressing annexin V and silencing of IL-13Rα2 augmented this cell death response (Fig. 5a). Interestingly, as seen with IL-13Rα2, the silencing of TMEM219 caused a similar significant increase in H2O2 sensitivity with significantly larger numbers of TMEM219 deficient cells expressing Annexin V compared with cells treated with scrambled siRNA controls (Fig. 5a). Similar results were seen in comparisons of macrophages from wild-type (WT) and TMEM219 null mice. As can be seen in Fig. 5b, the number of cells that were undergoing H2O2-induced apoptosis, as manifest by positive TUNEL staining, was significantly increased in cells from the TMEM219 null mice versus the controls. In addition, rChi3l1 inhibited H2O2-induced apoptosis in mice that contained normal levels of TMEM219 (Fig. 5b). In contrast, as previously shown for IL-13Rα2 (ref. 13), the anti-apoptotic effects of Chi3l1 were diminished in the absence of TMEM219 and this defect in Chi3l1-induced antiapoptosis could be rescued by the overexpression of TMEM219 (Fig. 5b). When viewed in combination, these in vitro studies demonstrate that, like IL-13Rα2, TMEM219 plays a critical role in the regulation of H2O2-induced cellular apoptosis.

To define the role of TMEM219 in vivo, we characterized the effects of 100% oxygen in WT, IL-13Rα2−/− and TMEM219−/− mice. After 72 h of exposure to hyperoxia, the mice were sacrificed and pulmonary cell death responses were evaluated using TUNEL staining. As previously reported15,16, hyperoxia caused a tissue cell death response in lungs from WT mice that were characterized by modest numbers of TUNEL-positive airway and alveolar epithelial cells, and the magnitude of this response was exaggerated in lungs from IL-13Rα2 null animals (Fig. 5c,d). In accord with the findings in the IL-13Rα2 null mice, a similar increase in airway and parenchymal epithelial cell apoptosis was seen in TMEM219 null animals (Fig. 5c,d). Accordingly, the level of BAL total protein (a measure of oxidant-induced lung injury) was also significantly increased in the IL-13Rα2−/− or TMEM219−/− mice compared with WT controls (Fig. 5e). In addition, with extended exposure to 100% oxygen, the survival of the IL-13Rα2−/− and TMEM219−/− mice was decreased compared with WT controls (Fig. 5f). These studies demonstrate that IL-13Rα2 and TMEM219 play similar roles as inhibitors of oxidant-induced acute lung injury.

Figure 3 | TMEM219 plays a critical role in Chi3l1-stimulated HB-EGF expression by epithelial cells and macrophages. (a,b) HB-EGF production by 1HAEo cells. 1HAEo cells were treated rhChi3l1 (500 ng ml−1) with (+) and without (−) siRNA silencing of IL-13Rα2 or IL-13Rα1 or TMEM219. The concentrations of HB-EGF were assessed by ELISA. (c) HB-EGF production by macrophages. Peritoneal macrophages were prepared from WT, IL-13Rα2 or TMEM219 null mutant mice and incubated with rmChi3l1 (500 ng ml−1) for 48 h. The levels of supernatant HB-EGF were assessed by ELISA. (d) Effects of anti-TMEM219 on HB-EGF production by 1HAEo cells. 1HAEo cells were stimulated for 48 h with rhChi3l1 (500 ng ml−1) in the presence and absence of TMEM219 neutralizing antibodies. In all panels, the levels of supernatant HB-EGF were assessed by ELISA. The values represent the mean ± s.e.m. of triplicate evaluations in a minimum of three separate experiments. **P<0.01; ***P<0.001; ns, non-significant by Student’s t-test.
function of IL-13Rα2, we initially evaluated the tissue responses in WT and TMEM219<sup>−/−</sup> mice after ovalbumin (OVA)-sensitization and challenge. These studies revealed augmented responses in TMEM219 null mice that were similar to what has been described in IL-13Rα2 null animals<sup>18</sup>. Specifically, the tissue infiltration of inflammatory cells, and the levels of BAL eosinophil recovery were significantly increased in TMEM219<sup>−/−</sup> mice compared with WT controls (Fig. 6a,b). The levels of BAL IL-4 and OVA-specific IgE were also significantly higher in IL-13<sup>Tg</sup> mice compared with WT or IL-13<sup>Tg</sup> null mutant mice (Fig. 6c–e). Interestingly, the levels of IL-1β were not similarly altered (Supplementary Fig. 7a).

Studies were next undertaken to determine if TMEM219 regulated the effects of IL-13. This was done by characterizing the effects of lung-targeted Tg IL-13 in WT and TMEM219 null mice. As shown in Fig. 6, there were impressive increases in IL-13-stimulated fibrotic and inflammatory responses in IL-13 Tg mice that contained null mutations of TMEM219 (IL-13 Tg/TMEM219<sup>−/−</sup> mice) compared with WT mice and IL-13 Tg mice with WT genetic backgrounds (Fig. 6f–h). In accord with these findings, the levels of total and active TGF-β1 in lungs from IL-13 Tg mice were significantly increased in the absence of TMEM219 (Supplementary Fig. 7b). In addition, IL-13-induced mucus metaplasia and BAL Muc5ac were also increased in the airways of IL-13 Tg/TMEM219<sup>−/−</sup> mice compared with WT or IL-13 Tg only controls (Fig. 6j). Lastly, in accord with the known importance of STAT6 signalling in mediating the tissue effects of IL-13 (refs 15,19), STAT6 activation in lungs from IL-13 Tg mice was also enhanced in mice with null mutations of TMEM219 (Fig. 6k). When viewed in combination, these studies demonstrate that, like IL-13Rα2 (ref. 18), TMEM219 is an important inhibitor of aeroallergen-induced Th2 inflammation and cytokine elaboration and IL-13-induced inflammation, fibrosis, mucus metaplasia and STAT6 signalling.

**TMEM219 contributes to the decoy effects of IL-13Rα2.**

The studies noted above demonstrate that TMEM219 inhibits IL-13-induced tissue responses in a manner that is similar to the decoy function attributed to IL-13Rα2. To address this possibility more formally, we first assessed the in vivo association of IL-13 and IL-13Rα2 in the presence and absence of TMEM219. In these studies we used Co-IP and IB assays with lung lysates from progeny of crosses of IL-13 Tg mice and TMEM219<sup>−/−</sup> mice. These studies highlighted decreased levels of binding between IL-13 and IL-13Rα2 in the lungs from IL-13 Tg mice that lacked TMEM219 compared with IL-13Tg with a WT background (IL-13Tg/TMEM219<sup>+/+</sup>) (Supplementary Fig. 8a). We also evaluated the ability of rIL-13 to bind to rIL-13Rα2 in the presence and absence of rTMEM219. These studies demonstrated that ability of rIL-13 to bind to rIL-13Rα2 was increased in the presence of rTMEM219 while rTMEM219 did not bind to rIL-13 (Supplementary Fig. 8bc). These findings demonstrate that TMEM219 facilitates IL-13-IL-13Rα2 binding and by so doing contributes to the decoy function attributed to IL-13Rα2.

**TMEM219 in melanoma metastasis and TGF-1 production in vivo.**

Recent studies from our laboratory and others have demonstrated that the metastasis of malignant melanocytes to the lung is...
evaluations in a minimum of three separate experiments. The values in Composite illustration taken from the noted three groups of mice (n = 5) and TMEM219+/− (n = 7) mice after exposure of 100% O2. The TMEM219−/− and IL-13Rα2−/− mice all had significantly decreased survival compared with WT mice (P < 0.01, log-rank Mantel–Haenszel test). (a) Representative of three separate experiments. (c) Composite illustration taken from the noted three groups of mice (N = 5) that behaved similarly. The values in b represent the mean ± s.e.m. of triplicate evaluations in a minimum of three separate experiments. The values in d,e represent the mean ± s.e.m. of evaluations in a minimum of five mice **P < 0.01; ***P < 0.001. NS, not significant by Student’s t-test.
mediated by an IL-13Rα2-dependent mechanism, which requires the production of TGF-β1 (refs 2,10,13). Thus, studies were undertaken to determine if TMEM219 plays a role in these responses. To accomplish this, we compared the metastasis of B16-F10 melanoma cells and the TGF-β1 that they induce in WT mice and TMEM219 null mice. Melanoma cell administration caused impressive levels of metastasis in lungs from WT mice and this metastatic response was markedly decreased in lungs from TMEM219−/− mice (Fig. 7a,b). In accord with the studies of Strober et al.2,10 melanoma metastasis was associated with significant increases in the levels of total and activated TGF-β1 in lungs from WT mice (Fig. 7c,d). Importantly, the levels of
TGF-β1 were significantly decreased in mice with null mutations of TMEM219 (Fig. 7cd). When viewed in combination, these studies demonstrate that, like IL-13Rα2, endogenous TMEM219 plays a critical role in pulmonary melanoma metastasis and the TGF-β1 elaboration that underlies this response.

Discussion

IL-13Rα2 is a 56 kDa glycoprotein that has engendered controversy based on its reported ability to act as a decoy as well as a signalling receptor2,8,13,17. To further understand the biology of IL-13Rα2 and shed light on this controversy, studies were undertaken to determine if this complex biology might be able to be explained, at least in part, by the ability of IL-13Rα2 to interact with and utilize binding partners in mediating ligand-induced effector responses. These studies demonstrate that IL-13Rα2 binds to TMEM219 at baseline and that this interaction is augmented in the presence of the IL-13Rα2 ligands Chi3l1 and IL-13. They also demonstrate that optimal Chi3l1 activation of MAPK and Akt signalling, inhibition of apoptosis, amelioration of oxidant injury, mediation of melanoma metastasis, stimulation of TGF-β1 elaboration during lung metastasis and inhibition of Th2 and IL-13-induced inflammation require both IL-13Rα2 and TMEM219.

To begin to address the possibility that IL-13Rα2 interacts with other proteins in mediating its effector responses, we initially employed Y2H screening to identify molecules that bind to IL-13Rα2. These studies identified TMEM219 as an IL-13Rα2 binding partner. This was followed by studies that demonstrated that IL-13Rα2 and TMEM219 co-immunoprecipitate and colocalize. Furthermore, reconstitution of purified TMEM219 into 13 nm nanodiscs and FA experiments confirmed the interaction of the TMEM219 nanodiscs and the purified ecto-IL-13Rα2 in the presence of Chi3l1. These observations allow for the interesting possibility that IL-13Rα2 may first bind its ligand, Chi3l1 or IL-13, and that this complex is then able to recruit TMEM219 to initiate signalling. Interestingly, both IL-13Rα2 and TMEM219 have short intracellular tails. This allows for the interesting hypothesis that IL-13Rα2 and TMEM219 behave like the α and β dimers of the T cell antigen receptor (TCR), which also have short intracytoplasmic tails and utilize other surface molecules like CD3 to transmit signals20,21. IL-13Rα2 and TMEM219 both exist in the cell membrane and in intracellular pools. Thus, additional investigation will be required to further define the mechanisms that IL-13Rα2 and TMEM219 use to transmit signals and the locations and constituents of this IL-13Rα2 and TMEM219 containing receptor complex.

Previous studies from our laboratory demonstrated that Chi3l1 binds to and signals via IL-13Rα2 (ref. 13). In these studies, it was appreciated that Chi3l1 interacts with IL-13Rα2 via different mechanisms to activate different downstream signalling pathways. Specifically, the Wnt/β-catenin pathway and the MAPK and AKT pathways were activated via mechanisms that were independent of and dependent on the 17 amino acid intracellular segment of IL-13Rα2, respectively13. The present studies confirm these observations by once again showing differences in the ways that IL-13Rα2 activates these signalling pathways. They also provide a potential mechanism for these differences by demonstrating that IL-13Rα2 activation of MAPK/Erk and Akt are dependent on TMEM219 while the Wnt/β-catenin pathway activation is mediated via an alternative mechanism. It will be interesting to determine if IL-13Rα2 is able to activate the Wnt/β-catenin pathway by itself or if it uses other binding partners to accomplish this event.

In keeping with its evolutionary conservation over species and time, Chi3l1 has been shown to regulate a number of
fundamental processes including the cell death responses, tissue oxidant injury, tumour metastasis and the induction of TGF-β1 and HB-EGF elaboration\(^{13,16,22-24}\). In accord with prior reports from our laboratory, these responses were mediated via pathways that required IL-13Rx2 (ref. 13). Importantly, each also required TMEM219 because phenocopies were seen in mice that lacked IL-13Rx2 or TMEM219 and cells in which each of these moieties was silenced. When viewed in combination, these studies demonstrate that signalling via the IL-13Rx2 and TMEM219 containing multimeric chitosome is an important event in these critical and diverse biologic responses. They also suggest that interventions that target IL-13Rx2, TMEM219 or other components of the chitosome might be useful in attempts to control these fundamental biologic events.

IL-13Rx2 was initially described as a decoy receptor that controls the tissue effects of IL-13. Subsequent studies demonstrated that IL-13Rx2 is a signalling receptor that mediates important biologic events\(^{25}\). In seeming support of the latter point of view, the present studies demonstrate, for the first time, that IL-13Rx2 utilizes TMEM219 as a partner in many of these responses. To address the possibility that TMEM219 also plays a role in the decoy function of IL-13Rx2, we compared the adaptive Th2- and IL-13-induced responses in WT mice and mice with null mutations of TMEM219. In accord with observations in IL-13Rx2 null mice\(^{18}\), we noted augmented aeroallergen-induced and IL-13-induced inflammation, cytokine, IgE, remodelling and signalling responses in mice that lacked TMEM219. Studies with pulmonary tissues and recombinant proteins also showed that TMEM219 contributes to optimal IL-13 binding to IL-13Rx2. These studies demonstrate that TMEM219 plays an important role in the decoy as well as the signalling properties of IL-13Rx2. They also contribute to the resolution of the controversy regarding the fundamental role of IL-13Rx2 by demonstrating that the decoy and signalling features of IL-13Rx2 are not mutually exclusive and that both are important features of this moiety.

The present studies demonstrate that mice that lack IL-13Rx2 or TMEM219 manifest similar increases in IgE production and Th2 cytokine elaboration and similar decreases in MAPK and Akt signalling and melanoma metastasis. They also demonstrate that TMEM219 and IL-13Rx2 bind to each other, that TMEM219 is required for optimal Chi3l1-induced, IL-13Rx2-dependent cell and tissue effects and that TMEM219 augments the binding of IL-13 to IL-13Rx2 without interacting directly with IL-13Rx1. As noted above, these findings demonstrate that TMEM219 plays a critical role in Chi3l1 signalling while simultaneously contributing to the decoy effects of IL-13Rx2. They also suggest that, in the absence of TMEM219, the local levels of free IL-13 are increased thereby augmenting IL-13 signalling via the canonical IL-13 receptor dimer (IL-13Rx1 and IL-4Rx2). This explains how an absence of TMEM219 induces tissue responses characterized by decreased MAPK/Erk and Akt signalling and increased Stat6 signalling. It also suggests that interventions that block the interaction of IL-13Rx2 and TMEM219 will be powerful regulators of inflammation, injury and or remodelling. It will be exciting to test these interventions in diseases and models of diseases that are characterized by Chi3l1 excess once they are developed.

The biology of TMEM219 has not been a topic of extensive investigation. The little that has been done has identified TMEM219 as a receptor for insulin-like growth factor binding protein-3 (IGFBP-3)\(^{25}\) and demonstrated that it contributes to biologic responses that are IGFBP3-dependent and -independent\(^{25,26}\). In these settings, TMEM219 has been shown to inhibit cell proliferation and induce cellular apoptosis, activate MAP kinases and induce angiogenesis\(^{25,27-30}\). Interestingly, the proapoptotic effects of TMEM219 that have been described to date stand in marked contrast to the IL-13Rx2 and TMEM219-mediated anti-apoptotic effects of Chi3l1 that are described in this report. In contrast, there are interesting similarities between the TMEM219-based activation of MAP kinases described in the literature and in this report. In addition, proangiogenic properties of Chi3l1 have also been described\(^{11}\). When viewed in combination, it is tempting to speculate that TMEM219 has ligand and cell context specific effector functions and that the presence of IL-13Rx2, and its ligands is a major determinant of the role of TMEM219 in a given setting. It is also tempting to speculate that the angiogenic effects of TMEM219 that have been described to date are mediated via its ability to interact with IL-13Rx2 and its ligands. Additional investigation will be required to address these speculations.

Prior studies from our laboratory and the present investigations demonstrate that IL-13Rx2 and TMEM219 play critical roles in melanoma metastasis\(^{13}\). In accord with the studies of Strober et al.\(^{21}\), that demonstrated that melanoma metastasis requires the induction of TGF-β1 in lungs from WT mice, the levels of TGF-β1 were significantly decreased in mice with null mutations of TMEM219 (Fig. 7c,d). In contrast, studies from our laboratory and others demonstrated that Tg IL-13 elicits tissue fibrosis via mechanisms that are dependent and independent of TGF-β1 (refs 32–34) and the present studies demonstrate that, in the absence of TMEM219, the levels of TGF-β1 and tissue fibrosis are increased. These findings are in accord with our prior demonstration that the levels of TGF-β1 are increased in IL-13 Tg mice with null mutations of IL-13Rx2 when compared with identical Tg mice on a WT background\(^{18}\). They are also in accord with our demonstration that TGF-β1 induction in IL-13 Tg mice is mediated by the canonical IL-13 receptor (IL-13Rx1-IL-4Rx2) while TGF-β1 induction in the setting of melanoma metastasis is mediated via an IL-13Rx2-dependent mechanism. When viewed in combination, these studies allow for the interesting speculation that the increased fibrosis that is seen in IL-13 Tg mice with null mutations of TMEM219 is due to the abrogation of IL-13Rx2 decoy function with heightened levels of free IL-13 and exaggerated IL-13-induced tissue effects including TGF-β1 elaboration. These studies also highlight the complex relationships between IL-13, the IL-13 receptors and TGF-β1 and the context-dependent way that TMEM219 contributes to these responses since it plays a critical role in TGF-β1 elaboration in the presence of metastasis but inhibits TGF-β1 in the presence of Tg (free) IL-13.

In summary, these studies demonstrate that TMEM219 binds IL-13Rx2 complexed with Chi3l1 and that the multimeric complex that is formed mediates diverse signalling and effector responses of Chi3l1. They also highlight the importance of TMEM219 in key Chi3l1-induced and IL-13Rx2-mediated biologic responses including the regulation of cellular apoptosis, oxidative tissue injury, allergic inflammation, tumour metastasis and TGF-β1 and HB-EGF elaboration. Additional investigation of TMEM219, IL-13Rx2, Chi3l1 and the multimeric chitosome complex that they form is warranted in health and disease.

**Methods**

**Mice.** C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and were housed at the Yale University and Brown University animal facilities. IL-13 Tg mice were maintained and characterized in our laboratory as previously described\(^{13}\). IL-13Rx2 null mice (IL-13Rx2\(^{-/-}\)) were purchased from The Jackson Laboratory and backcrossed to C57BL/6 background (> 10 generations). TMEM219 null mice (TMEM219\(^{-/-}\)) were purchased from the Knock Out Mice Project (KOMP) Repository at University of California, Davis. The mice that were generated were backcrossed to C57BL/6 background for >10 generations and age- and sex-matched 6-8-weeks-old mice were used for proposed experiment. All murine procedures were approved by the Institutional Animal Care and Use Committees at Yale and Brown Universities.
Y2H screening

The full-length human IL-13Rα2 gene was amplified by PCR from human lung cDNA using the following primers (forward, 5′-AAG GGT TTC CAT ATG GCT GCA GCT ACG-3′; reverse, 5′-ATG TGA TAT CTC GAG (XhoI) TCT TCA TGT ACG GCA GAA-3′). The IL-13Rα2 DNA was cloned into the Y2H BD vector at the NdeI and SalI sites. The precipitates were then washed three times according as per the manufacturer’s instruction. The precipitates were then resuspended in 50 mM sodium citrate, 0.05% Tween 20, pH 6. 

**Co-localization with double-label IHC.** To localize the expression of IL-13Rα2 and TMEM219, double-label IHC was undertaken with antibodies against both IL-13Rα2 (as a bait) and library plasmids. A total of 2 × 106 of these double transformants were then grown on dropout medium deprived of tryptophan, leucine, histidine and adenine (Trypt-Leu-His-Adr) to select for protein–protein interactions. Transformants that grew were assayed for g-galactosidase activity on 5-bromo-4-chloro-3-indolylphosphate (X-Gal) plates according to the manufacturer’s instructions (Clontech, Palo Alto, CA, USA). Plasmids were extracted from blue yeast clones and were then transfected into Escherichia coli DH5α. Bacteria were grown on medium with ampicillin to select for bacteria containing library plasmids. The plasmid DNA was then isolated and sequenced with a primer reverse complement sequence of the pGBD-T7 vector as a forward and reverse primer, respectively. Homology searches were performed using the National Center for Biotechnology Information database with the Blast program. All positive constructs were rescreened for their ability to grow on complete dropout medium when transfected together with IL-13Rα2 null, TMEM219 null and empty pGBK7 plasmid to verify potential interactions and to eliminate false positives.

**Preparation and stimulation of peritoneal macrophages.** Peritoneal macrophages were isolated from 7-week-old IL-13Rα2 null, TMEM219 null and WT mice, which had been injected intra peritoneally 3 days earlier with 3 ml of thiglycollate. The collected cells were washed twice with PBS and then cultured in DMEM containing 10% heat-inactivated FBS, 1 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin at a density of 2 × 106 cells/ml. Cell cultures were then split and incubated in 5% CO2 incubator. Then, the cultures were washed twice with PBS to remove non-adherent cells before the addition of 1 ml of fresh medium. Antibodies against F4/80 (565410, PharMingen, San Diego, CA, USA) and FACS analysis on a FACSCalibur apparatus (Becton Dickinson, San Jose, CA, USA) were used to assess cellular purity. In select experiments macrophages were incubated with IL-13, rCh311 or vehicle control. Experiments with rCh311 were done in the absence of serum.

**siRNA silencing of IL-13Rα2 and TMEM219.** siRNAs specific for human IL-13Rα2 and TMEM219 (Santa Cruz) were used to decrease the levels of mRNA encoding IL-13Rα2 and TMEM219 as per the manufacturer’s instructions. Cells were seeded on six well plates and transfected the next day with IL-13Rα2, TMEM219 or control siRNAs. The cells were collected at the indicated time points and subjected to real-time (RT)-PCR or western blot evaluations. Quantitative RT-PCR and western blot evaluations using anti-IL-13Rα2 (1/500 dilution, AF146, Biotechnology Inc.) antibodies demonstrated that these siRNA had over 90% of silencing efficiencies for IL-13Rα2 and TMEM219 (Supplementary Fig. 4).

**Generation of monoclonal antibodies against TMEM219.** Anti-mouse TMEM219 monoclonal antibody was generated using synthetic 10-mer peptides (RPEFHWSRTR corresponding to 230–239 region of TMEM219) (Abmart, Shanghai, China) and further purified using Montage Antibody Purification Kit (EMD Millipore). The purified antibodies demonstrated that these siRNA had over 90% of silencing efficiencies for IL-13Rα2 and TMEM219 (Supplementary Fig. 4).
evaluations were undertaken in the presence and absence of rmChi3L1 (R&D Systems, 500 ng ml⁻¹), after treatment with TMEM219 and or control siRNA (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) or after transfection with TMEM219 or control cDNA. The mRNA cell death response was evaluated in WT, TMEM219⁻/⁻ and IL-13Rx2⁻/⁻ mice after exposure to 100% oxygen or room air for up to 3 days as described previously.¹⁹

Data availability. All data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon request.

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C.-M.L, C.G.L and J.A.E. designed the studies. C.-M.L., C.H.H., A.M.N., C.G.L. and J.A.E. performed the experiments. A.M.N., M.L.N. and Y.M. designed and ran the biochemical evaluations.

Additional information
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