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Aditya Kotha
Virginia Commonwealth University

Jordan M. Dailey
Virginia Commonwealth University

Aslamuzzaman Kazi
Virginia Commonwealth University

Said Sebti
Virginia Commonwealth University

John J. Ryan
Virginia Commonwealth University

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Aditya Kotha[1], Jordan M. Dailey[1], Aslamuzzaman Kazi[2], Said Sebti[2], and John J. Ryan[1]

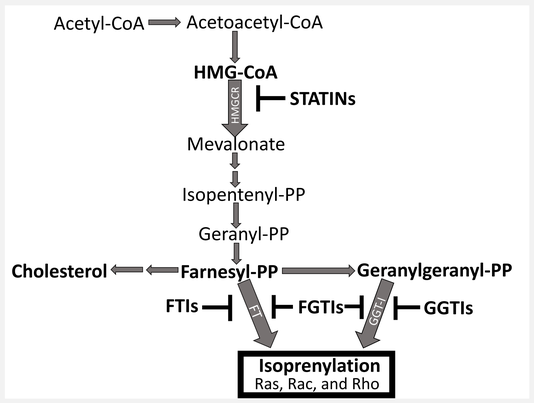
[1]Department of Biology, Virginia Commonwealth University, Richmond, VA 23284
[2]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond VA USA 23298

Abstract

Allergic disease is driven by cell signaling cascades that activate immune cells. One key player is the mast cell, which is activated by IgE antibodies signaling through the high affinity IgE receptor, FcεRI. Therefore, targeting FcεRI-mediated cascades can offer novel treatments for allergic disease. Statins have been demonstrated to reduce the severity of asthma, a common allergic airway disease. Statins are an FDA approved class of drugs with the intended purpose of lowering blood cholesterol. We previously found that while statins inhibit mast cell function in allergic disease, these anti-inflammatory effects vary widely amongst differing mouse strains and human donors, suggesting genetic variability. This project sought to overcome statin resistance by acting "downstream" in the cholesterol synthesis pathway on protein isoprenylation pathways. The logic is that isoprenylated proteins are critical for FcεRI signaling, thus blocking this step of protein modification should reduce FcεRI-mediated mast cell function. The novel drug FGTI-2734 was used to suppress the isoprenylation enzymes farnesyl transferase and geranylgeranyl transferase. FGTI-2734 reduced IgE-mediated mast cell degranulation and cytokine and chemokine secretion. Additional work found that both transferases must be targeted to produce these anti-inflammatory effects. Furthermore, we revealed that the K-Ras protein is an isoprenylation target that is essential for IgE-mediated mast cell function. Collectively, these studies demonstrate the translational potential of the novel drug FGTI-2734 and suggest it acts by suppressing isoprenylation of proteins critical for mast cell function, including K-Ras.

Results

Fig. 1: Mechanism of action of statins and FGTI-2734. The target of statins is HMG-CoA reductase (HMGCR), which is the rate limiting step of the cholesterol synthesis pathway. Targeting HMGCR will inhibit cholesterol and isoprenoid synthesis. FGTI-2734 inhibits the transferases that will attach a FPP or GPP isoprenoid to a protein.



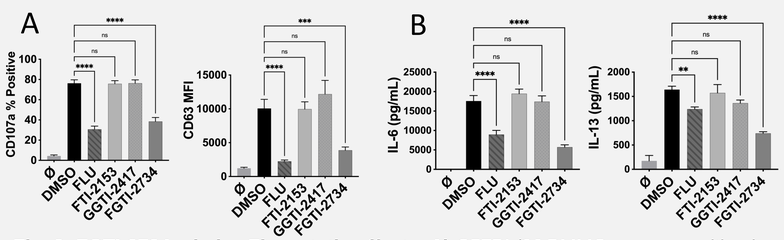


Fig. 2: FGTI-2734 mimics Fluvastatin effects. A) C57BL/6J BMMCs were sensitized with IgE and treated with fluvastatin (5μM), FTI-2717 (5μM), GTI-2418 (5μM), FGTI-2734 (5μM), or vehicle control for 24 hours, then activated by IgE XL for 15 minutes and degranulation markers CD107a and CD63 were measured by flow cytometry. **B)** BMMCs were treated as in (A) but activated for 16 hours and cytokines were measured in culture supernatant by ELISA.

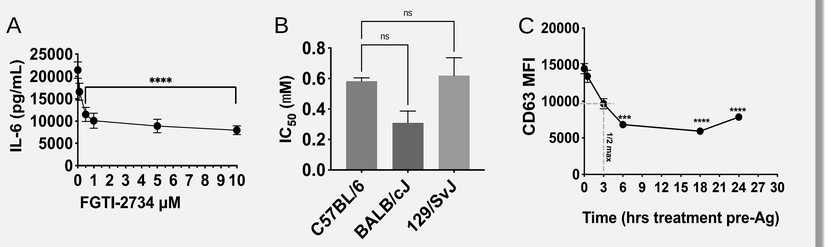


Fig. 3: FGTI-2734 is dose and time dependent. A) BMMCs were treated with increasing doses of FGTI-2734 for 24 hours prior to IgE XL for 16 hours. Cytokines were measured by ELISA. **B)** BMMCs derived from C57BL/6J or statin-resistant BALB/c and 129/SvJ mice were treated as in (A) and IC50 values for suppressing IL-6 production were calculated. **C)** BMMCs were treated with FGTI-2734 (5μM) for the indicated times before IgE XL for 15 minutes. CD63 expression was measured by flow cytometry and $t_{1/2}$ for inhibition was calculated.

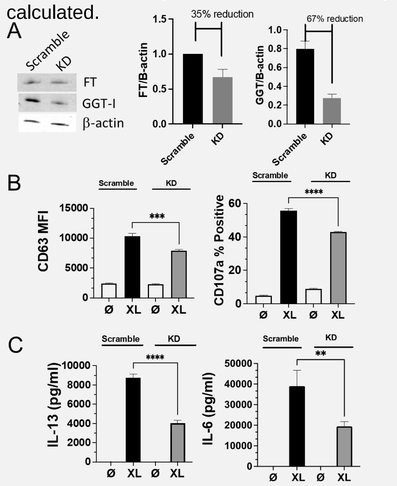


Fig. 4: GGT-1 and FT siRNA targeting reduce IgE-mediated degranulation and cytokine production. C57BL/6J BMMCs were transfected with siRNAs targeting GGT-1, FT, or with scrambled control siRNAs. **A)** Cell lysates were assessed by Western blot for GGT-1 and FT expression, which was normalized to actin loading control for quantification. **B)** Transfected cells were activated by IgE XL for 15 minutes and degranulation markers CD63 and CD107a were measured by flow cytometry. **C)** Transfected cells were activated by IgE XL for 16 hours, and cytokines were measured by ELISA. Data shown are from 2 experiments using 6 samples.

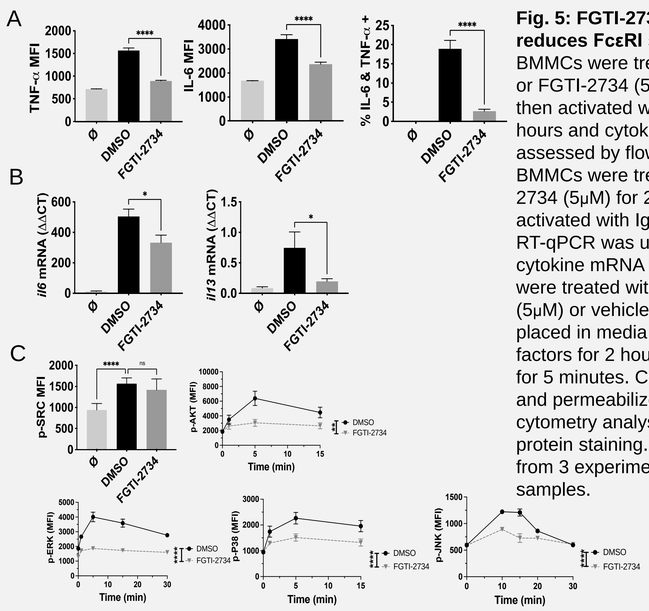


Fig. 5: FGTI-2734 treatment reduces FcεRI signaling. A) BMMCs were treated with vehicle or FGTI-2734 (5μM) for 24 hours then activated with IgE XL for 5.5 hours and cytokine production was assessed by flow cytometry. **B)** BMMCs were treated with FGTI-2734 (5μM) for 24 hours and activated with IgE XL for 4 hours. RT-qPCR was used to measure cytokine mRNA levels. **C)** BMMCs were treated with FGTI-2734 (5μM) or vehicle for 24 hours and placed in media without growth factors for 2 hours before IgE XL for 5 minutes. Cells where fixed and permeabilized prior to flow cytometry analysis of phospho-protein staining. Data shown are from 3 experiments using 3 samples.

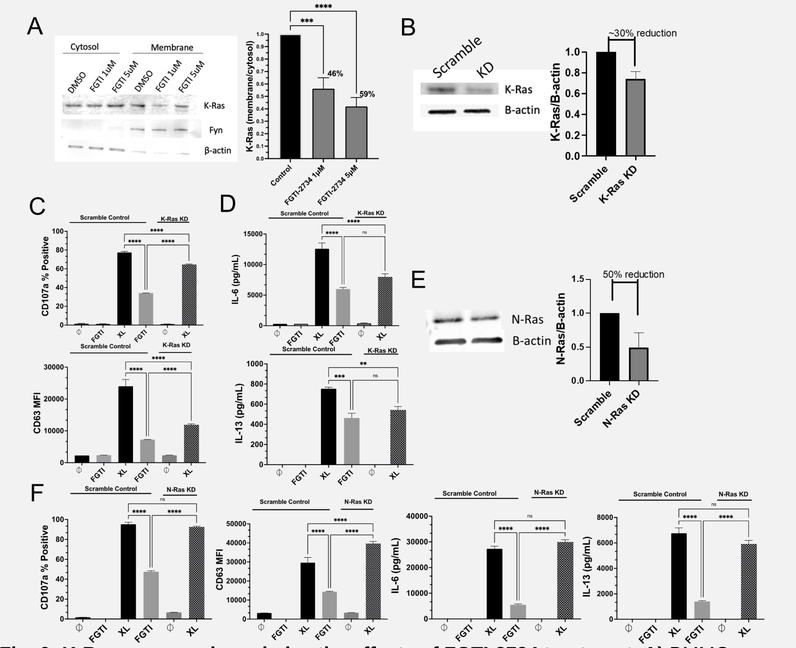


Fig. 6: K-Ras suppression mimics the effects of FGTI-2734 treatment. **A)** BMMCs were treated with either FGTI-2734 or vehicle control for 24 hours and lysed. Membrane and cytosolic fractions were assessed for K-Ras by Western blot. Fyn, a membrane-associated protein that is not isoprenylated, and actin, a cytosolic protein, were used to determine the effectiveness of cell fractionation. **B)** BMMCs were transfected with siRNA targeting K-Ras as in Fig. 4. Lysates were assessed for K-Ras expression by Western blot. **C)** Cells from (B) were activated by IgE XL for 15 minutes and degranulation markers CD107a and CD63 were measured by flow cytometry. **D)** Cells from (B) were activated by IgE XL for 16 hours and cytokines were measured by ELISA. **E)** BMMCs were transfected with siRNA targeting N-Ras as in Fig. 4. Cell lysates were assessed for N-Ras expression by Western blot. IgE XL-induced degranulation and cytokine production were assessed as described for K-Ras targeting. Data shown are from 3 individual experiments in (A). Parts (B-E) are representative of at least 2 independent experiments. P values were calculated by ANOVA.

Conclusions & Future Directions

- FGTI-2734 is a dual isoprenylation inhibitor that mimics Fluvastatin's effects on decreasing IgE-mediated mast cell activation and function.
- FGTI-2734 effects are dependent on dose and time to affect IgE-mediated mast cell activation.
- FGTI-2734 is able to reduce IgE-mediated activation and FcεRI signaling in mouse strains including 129/Svj mouse strain that is resistant to statin medications.
- Inhibiting isoprenylation cascades affects K-Ras protein and K-Ras signaling but not N-Ras protein nor N-Ras signaling.

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