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# Nrf2 activation via *Keap1* deletion or Sulforaphane treatment reduces Ova-induced sinonasal inflammation

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## To the editor:

The human airway plays an essential role in oxygen exchange and is subjected to airborne environmental, allergen, and infectious exposures. These agents incite oxidative stress either directly or indirectly through recruitment of inflammatory cells which release reactive oxygen species[1]. Oxidative stress has been linked to disease severity of lower airway chronic inflammatory disorders such as asthma and COPD, however, its role in upper airway (sinonasal) chronic inflammatory disorders is less clear[1–2]. Interestingly, increased expression of cytoprotective enzymes has been reported in chronic rhinosinusitis (CRS) suggesting that oxidative stress may play a role in CRS pathophysiology[3].

Nuclear erythroid 2 p45-related factor (Nrf2) is a transcription factor that upon activation invokes an anti-oxidant response pathway via nuclear translocation and up-regulation of cytoprotective genes[4]. Disruption of the Nrf2 pathway in mice has been reported to enhance susceptibility to Ovalbumin (Ova)-induced asthma[5]. Interestingly, activation of Nrf2 has recently been reported to stabilize sinonasal epithelial barrier function *in vitro*[4, 6]. We sought to determine whether sinonasal barrier stabilization through Nrf2 pathway activation could stabilize barrier function *in vivo* and reduce allergen-induced inflammation in a mouse model of rhinosinusitis.

Under normal circumstances, Nrf2 is sequestered in the cytoplasm and inhibited through binding Kelch-like ECH-associated protein 1 (Keap1)[7]. Disruption of this interaction results

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Author Contributions

Conceptualization and experimental design, N.R.L., A.T., A.L., V.S., S.B., M.R.; performed experiments and data collection, A.T., M.M., T.S., M.C., A.D., M.Z., A.H.H.; manuscript and figure preparation, N.R.L., A.T., S.B., M.R.; funding acquisition, M.R.

Declaration of Interests

N.R.L. is a patent co-inventor for methods treating vascular barrier dysfunction licensed to Navigen Pharmaceuticals which is unrelated to the Nrf2 pathway. N.R.L. holds a small amount of stock in Navigen Pharmaceuticals which is currently of no value. N.R.L. is a consultant for Cooltech Inc. The remaining authors declare no competing financial interests.

in Nrf2 activation and nuclear translocation. Therefore we tested whether Nrf2 activation via tamoxifen inducible *Keap1*$^{flox/flox;\ ER\text{-}cre}$ mice may reverse Ova-induced sinonasal barrier dysfunction. Indeed, deletion of *Keap1* significantly reduced sinonasal barrier breakdown as assessed by serum albumin accumulation in lavage fluid and zonula occludens (ZO-1) cell surface localization on immunofluorescence (Figure 1a-b). Furthermore, genetic activation of the Nrf2 pathway through *Keap1* deletion significantly reduced Ova-induced accumulation of eosinophils (Figure 1c-d) and goblet cell hyperplasia (Figure 1e-f). Details regarding the materials and methods may be found in this article's online supporting information (Data S1).

Activation of Nrf2 and stabilization of the sinonasal epithelial barrier can also be achieved through stimulation with the small molecule Sulforaphane (SFN)[4]. We therefore hypothesized that SFN treatment may limit Ova-induced sinonasal barrier disruption and sought to assess pre-clinical therapeutic potential. Wildtype mice were subjected to the Ova-induced model of chronic sinonasal inflammation and treated with SFN at a dose found to increase Nrf2 downstream target expression (Figure S1-S2). SFN administration significantly reduced Ova-induced accumulation of serum albumin in nasal lavage fluid as well as disruption of cell surface ZO-1 localization (Figure 2a-b). SFN administration also reduced disruption of cell surface epithelial cadherin (E-cadherin) localization although this improvement appeared to a lesser degree than ZO-1 (Figure 2c). SFN administration was also found to reduce eosinophil accumulation in sinonasal tissue (Figure 2d-e). Furthermore, Nrf2 activation via SFN treatment reduced eotaxin-1 levels in the nasal lavage fluid of these mice (Figure 2f).

These data demonstrate that Nrf2 activation via genetic and therapeutic approaches can stabilize barrier function and reduce sinonasal inflammation in a mouse model of rhinosinusitis. The Nrf2 pathway therefore represents a potential therapeutic target in CRS and chronic sinonasal inflammatory disorders[8]. These results are in line with previous reports in the lower airway that place Nrf2 activity as a key regulator of allergen-induced inflammation[5, 9]. Interestingly, while activation of Nrf2 through either *Keap1* deletion and SFN treatment profoundly reduced sinonasal barrier dysfunction *in vivo* (Fig 1a, Fig 2a), the effect on eosinophil accumulation in *Keap1* tamoxifen-inducible knockout mice was much less than SFN treatment (Fig 1d, Fig 2e). The explanation for this finding is unknown, but may perhaps be related to incomplete *Keap1* knockout in this tamoxifen-inducible system.

It remains unknown the mechanism whereby Nrf2 activation stabilizes sinonasal barrier function and promotes cell surface localization of ZO-1 and the adherens junction protein E-cadherin. A potential explanation given the known function of Nrf2 in a cytoprotective response includes an overall reduction of oxidative stress. If this were the sole mechanism, one may therefore hypothesize that any pathway or means in reduction of oxidative stress may have a similar effect in this mouse model of rhinosinusitis. Additional possibilities include an undefined effect on cell junction protein cell surface localization or an indirect mechanism. Previous studies *in vitro* have demonstrated that SFN can stabilize human sinonasal epithelial cell dysfunction in the presence of multiple stimuli including particulate matter, cigarette-smoke extract, and house dust mite antigen[4, 6]. Future studies are necessary to clarify the mechanism whereby Nrf2 stabilizes barrier function.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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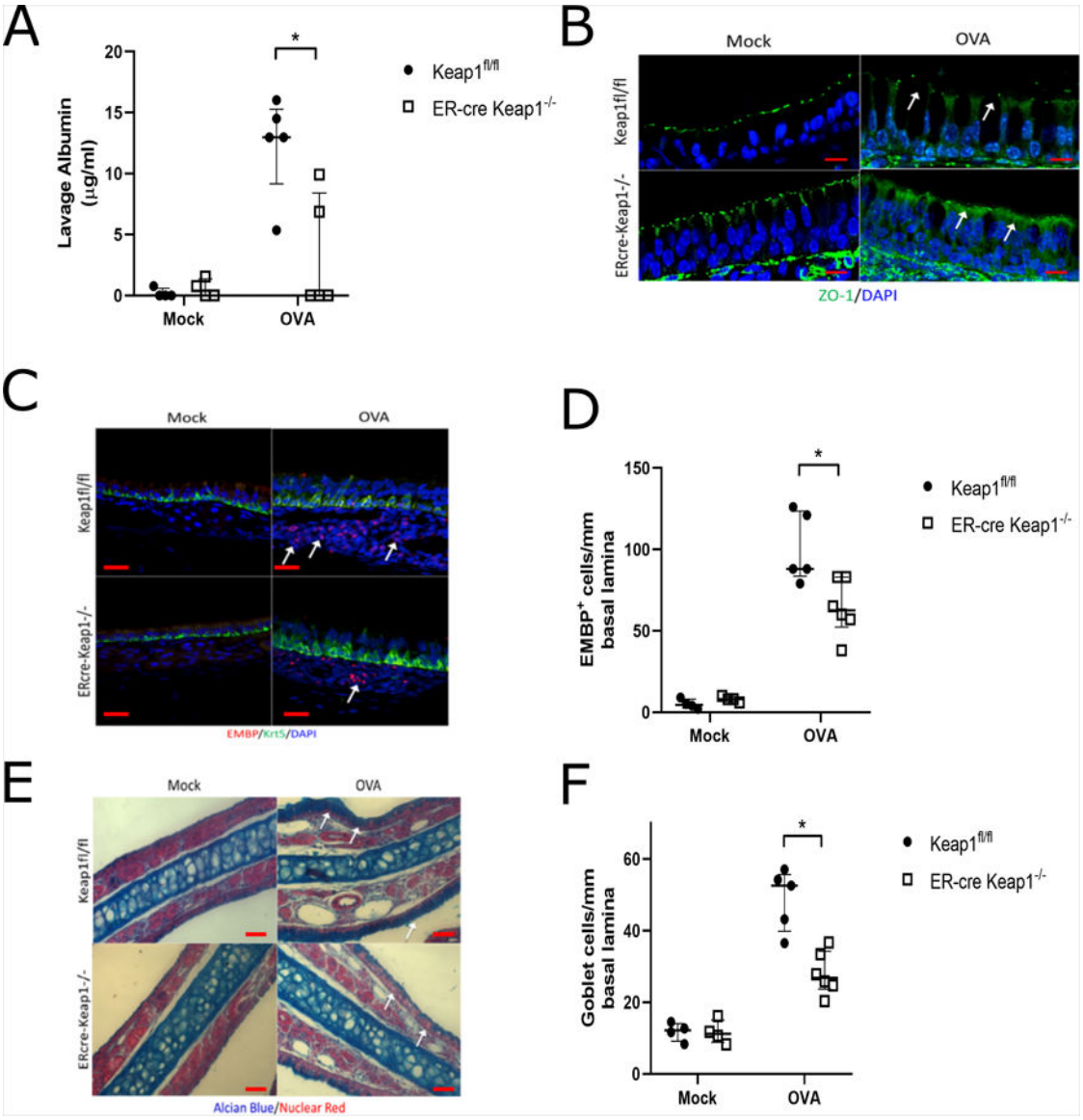


**Figure 1.**

*Keap1*$^{-/-;\ \text{ER-cre}}$ mice demonstrate reduced barrier dysfunction in response to chronic Ova exposure. (A) Analysis of nasal lavage fluid for serum albumin (n= 4–6 per group). Mock is vehicle control. (B) Immunofluorescence for ZO-1 (green) demonstrates reduced barrier disruption. DAPI (blue). Images are at 100x magnification and scale bar is 10µm. (C) Immunofluorescence for eosinophil major basic protein (EMBP, red) and Keratin-5 (Krt5, green). White arrows indicate areas of increased eosinophil accumulation. Images are at 40x magnification and scale bar is 20µm. (D) Eosinophil counts per mm of basal lamina from imaging of the entire nasal septum (n= 4–6 per group). Goblet cell via Alcian blue stain (E-F) counts per mm of basal lamina (n= 4–6 per group). DAPI (blue). Images are at 20x magnification and scale bar is 50µm. * $P$<0.05, ** $P$<0.01, *** $P$<0.001, **** $P$<0.0001 Data are represented as median with interquartile ranges.

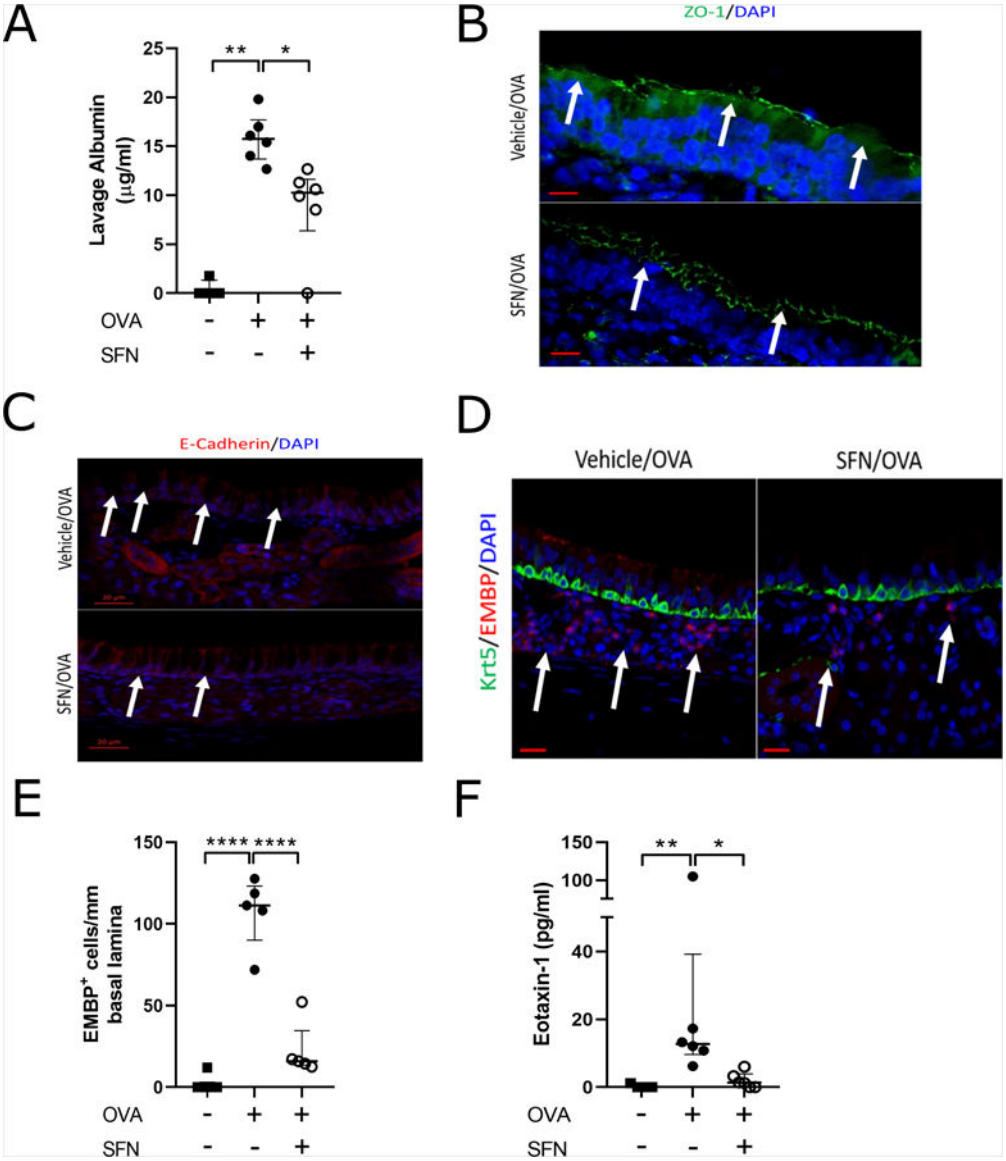


**Figure 2.**

Nrf2 activation via Sulforaphane treatment reduces Ova-induced sinonasal barrier dysfunction. (A) Analysis of nasal lavage fluid for serum albumin (n= 4–6 per group). (B) Immunofluorescence for ZO-1 (green) demonstrates reduced barrier disruption. Images are at 100x magnification and scale bar is 10µm. (C) Immunoflourescence for E-cadherin (red) demonstrates reduced barrier disruption. Images are at 25x magnification and scale bar is 20µm. (D) Immunofluorescence for eosinophil major basic protein (EMBP, red) and Keratin-5 (Krt5, green). White arrows indicate areas of increased eosinophil accumulation. Images are at 40x magnification and scale bar is 20µm. (E) Eosinophil counts per mm of basal lamina from imaging of the entire nasal septum (n= 4 per group). (F) Analysis of nasal lavage fluid via ELISA for eotaxin-1 (n= 4–6 per group). DAPI (blue) * $P$<0.05, ** $P$<0.01, **** $P$<0.0001, Data are represented as median with interquartile ranges.