

EDITORIAL

NRF2 Controls the Competitive Fitness of Squamous Epithelial Cells in the Mouse Esophagus

In the human esophagus, somatic cancer-associated mutations can occur within distinct clonal populations, some of which are selected over time.¹ In mice in which Cre-mediated deletion of the transcription factor NRF2 (nuclear factor erythroid 2-related factor) was designed to yield approximately equal levels of esophageal epithelial cells with *Nrf2* deletion and those with *Nrf2* intact, the *Nrf2*-deleted cells were eliminated rapidly on exposure to a chemical carcinogen,² suggesting a similar selection process. Functionally, NRF2 regulates stress responses and is itself regulated at the protein level by KEAP1 (Kelch-like ECH-associated protein 1), which promotes NRF2 degradation. Global *Keap1* deletion leads to hyperproliferation and hyperkeratosis in the esophageal squamous epithelium, whereas deletion of *Nrf2* along with *Keap1* completely rescues the phenotype.

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Hirose et al³ addressed the selective extinction of *Nrf2*-deleted cells in the esophageal epithelium by investigating whether cells with higher NRF2 levels had a competitive advantage over cells with normal NRF2 levels. To test this, the authors activated NRF2 in a subgroup of esophageal squamous epithelial cells by tamoxifen-induced recombination using *K5Cre^{ERT2};Keap1^{floxB/floxB}* mice. Expression of NRF2 in esophageal epithelial cells following conditional *Keap1* deletion (*Keap1*-cKO mice) was assessed by staining for the NRF2 transcriptional target NQO1. Mild hyperkeratosis was observed in the esophagus of these *Keap1*-cKO mice, in contrast to severe hyperkeratosis seen in global *Keap1* deletion. Interestingly, KEAP1-deleted cells (ie, NQO1 positive/NRF2-high) and KEAP1-normal cells (NRF2-normal) were committed to different fates, with the former being hyperproliferative and detached from the basement membrane for terminal differentiation and the latter undergoing compensatory hyperproliferation. As a result, KEAP1-deleted cells were outcompeted by KEAP1-normal cells and mostly eliminated from the esophageal epithelium.

Cell competition was further tested in *K5Cre^{ERT2};Keap1^{floxB/floxB};Nrf2^{SA/SA}* (*Keap1* cKO::Nrf2^{SA} mice), in which a knock-in mutation prevents degradation of NRF2 by KEAP1 and another protein, β -transducin repeat containing 21 protein (β TRCP). Because the *Nrf2^{SA}* mutation was global, *Keap1*-cKO::Nrf2^{SA} mice had higher levels of NQO1 (suggesting stronger NRF2 activation) than *Keap1*-cKO mice, and consistent with the prior results, the disappearance of NQO1-positive cells was even more rapid in *Keap1*-cKO::Nrf2^{SA} mice than in *Keap1*-cKO mice. Although replicative stress and DNA damage took place in proliferating KEAP1-normal epithelial cells, inflammatory cells also migrated to the submucosal and intraepithelial layers,

particularly in the regions surrounding the KEAP1-normal epithelial cells. Furthermore, *Keap1*-cKO mice developed many more chemically induced tumors than wild-type mice, and 26/27 of these tumors (96%) were KEAP1 positive. These data suggest that even though KEAP1-deleted cells lost the competition with KEAP1-normal cells, KEAP1-deleted cells still contributed to tumor formation of KEAP1-normal cells; potential mechanisms include residual inflammation and compensatory growth pressure resulting from the KEAP1-deleted cells that promoted proliferation and carcinogenesis in the KEAP1-normal cells or the generation of toxic metabolites, paracrine signals, or mechanical stress by KEAP1-deletion.³ Notably, NRF2 activation caused inflammation by compromising the epithelial barrier in the mouse skin, so this may also play a role in the esophageal epithelium.⁴

Manipulation of the competitive fitness of various cell populations may have important implications for esophageal cancer prevention and therapy. For example, a recent study using a chemical carcinogenesis model demonstrated that *Notch1*-inactivating mutations conferred a competitive advantage in the histologically normal esophageal epithelium and resulted in the elimination of microtumors.⁵ The study by Hirose et al³ implicates NRF2 signaling as another potential target regulating clonal selection in esophageal squamous epithelial cells. In human esophageal squamous cell carcinoma, NRF2 may be mutated, overexpressed, and hyperactive, whereas KEAP1 mutations are much less frequent. In those cancers with hyperactivating NRF2 mutations, NRF2-normal cells might be induced to outcompete neighboring NRF2-high cells before treatment with chemotherapy and radiation. Moderately high NRF2, which can be achieved through treatment with chemical NRF2 activators, but not high NRF2, would seem most desirable for esophageal cancer prevention.

Nevertheless, this study raises new questions about how NRF2 regulates homeostasis and carcinogenesis in the esophageal epithelium. For example, what molecules are essential to enhance the competitive fitness of KEAP1-normal/NRF2-normal cells? Do cells with activating mutations in *NRF2* have different fitness than wild-type NRF2-high cells when competing against KEAP1-normal cells/NRF2-normal cells? Is there a way to optimize NRF2 activity in the esophageal epithelium to a favorable level? Because the normal human esophagus is not keratinized like the mouse esophagus and hyperkeratosis is rarely seen in the human esophagus, are the findings in this study directly applicable to human esophagus? Further studies along these directions will be of great interest to those studying esophageal biology and cancer.

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136 Q6 References

- 137 1. Martincorena I, Fowler JC, Wabik A, et al. Somatic
 138 mutant clones colonize the human esophagus with age.
 139 Science 2018;362:911–917.
- 140 2. Horiuchi M, Taguchi K, Hirose W, et al. Cellular Nrf2 levels
 141 determine cell fate during chemical carcinogenesis in
 142 esophageal epithelium. Mol Cell Biol 2021;41:e00536-20.

3. Hirose W, Horiuchi M, Li D, et al. Selective elimination of
 176 NRF2-activated cells by competition with neighboring
 177 cells in the esophageal epithelium. Cell Mol Gastro-
 178 enterol Hepatol 2022;XX:XXX–XXX. 179
4. Schafer M, Willrodt AH, Kurinna S, et al. Activation of
 180 Nrf2 in keratinocytes causes chloracne (MADISH)-like
 181 skin disease in mice. EMBO Mol Med 2014;6:442–457. 182
5. Colom B, Herms A, Hall MWJ, et al. Mutant clones in
 183 normal epithelium outcompete and eliminate emerging
 184 tumours. Nature 2021;598:510–514. 185

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Conflicts of interest

The authors disclose no conflicts.

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