cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

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 al[3] 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 (βTRCP). 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 al[3] 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.

ZHAOHUI XIONG, PhD
Cancer Research Program
Julius L. Chambers Biomedical Biotechnology Research Institute
North Carolina Central University
Durham, North Carolina

XIAOXIN CHEN, MD, PhD
Cancer Research Program
Julius L. Chambers Biomedical Biotechnology Research Institute
North Carolina Central University
Durham, North Carolina
Center for Gastrointestinal Biology and Disease
Division of Gastroenterology and Hepatology
Department of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

References

1. Martincorena I, Fowler JC, Wabik A, Lawson ARJ, Abascal F, Hall MWJ, Cagan A, Murai K, Mahbubani K, Stratton MR, Fitzgerald RC, Handford PA, Campbell PJ, Saeb-Parsy K, Jones PH. Somatic mutant clones colonize the human esophagus with age. Science 2018;362:911–917.
2. Horiuchi M, Taguchi K, Hirose W, Tsuchida K, Suzuki M, Taniyama Y, Kamei T, Yamamoto M. Cellular Nrf2 Levels Determine Cell Fate during Chemical Carcinogenesis in Esophageal Epithelium. Mol Cell Biol 2021;41:e00536-20.
3. Hirose W, Horiuchi M, Li D, Motoike IN, Zhang L, Nishi H, Taniyama Y, Kamei T, Suzuki M, Kinoshita K, Katsuoka F, Taguchi K, Yamamoto M. Selective Elimination of NRF2-Activated Cells by Competition With Neighboring Cells in the Esophageal Epithelium. Cell Mol Gastroenterol Hepatol 2023;15:153–178.
4. Schäfer M, Willrodt AH, Kurinna S, Link AS, Farwanah H, Geusau A, Gruber F, Sorg O, Huebner AJ, Roop DR, Sandhoff K, Saurat JH, Tschachler E, Schneider MR, Langbein L, Bloch W, Beer HD, Werner S. Activation of Nrf2 in keratinocytes causes chloracne (MADISH)-like skin disease in mice. EMBO Mol Med 2014;6(4):442-57.
5. Colom B, Herms A, Hall MWJ, Dentro SC, King C, Sood RK, Alcolea MP, Piedrafita G, Fernandez-Antoran D, Ong SH, Fowler JC, Mahbubani KT, Saeb-Parsy K, Gerstung M, Hall BA, Jones PH. Mutant clones in normal epithelium outcompete and eliminate emerging tumours. Nature 2021;598:510–514.

Correspondence
Address correspondence to: Xiaoxin Chen, MD, PhD, Cancer Research Program, Julius L. Chambers Biomedical Biotechnology Research Institute, North Carolina Central University, 700 George Street, Durham, North Carolina 27707. e-mail: lchen@nccu.edu.

Conflicts of interest
The authors disclose no conflicts.

Funding
Xiaoxin Chen's laboratory is supported by the National Institutes of Health (R01 CA244236, R01 AA030026, U54 CA156735, U54 MD012392).


Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2022.10.010