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EDITORIAL

A Balancing Act: GRHL3 Limits WNT Signaling to Promote Tissue Homeostasis in the Esophageal Epithelium

Tissue homeostasis requires a balance between cellular proliferation and differentiation, and its disruption often manifests in disease. Understanding the tight regulation between cellular proliferation and differentiation holds promise to uncover innovative approaches to treating diseases in which the balance is altered, including cancer. Grainy head like 3 (GRHL3) is a member of a family of transcription factors highly conserved from *Drosophila* through humans.[1] Previous studies revealed critical roles for GRHL3 in epidermal development and skin barrier formation showing that GRHL3 is crucial for maintaining epidermal tissue homeostasis.[2–4] In GRHL3-deficient skin and oral epithelium, hyperproliferation ensues, rendering these tissues highly susceptible to chemically induced carcinogenesis.[5,6] Notably, tissue-specific pathways govern GRHL3's tumor suppressive functions in these tissues, with malignancy mediated by a GRHL3/PTEN/AKT proto-oncogene axis in skin[6] and a GRHL3/GSK3ß/c-Myc axis in the oral epithelium.[5] The function of GRHL3 in the esophageal epithelium had not been explored. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Georgy et al[7] discover a novel GHRL3 tumor suppressive mechanism active in the esophagus by which loss of GRHL3 disrupts the proliferation/differentiation balance in stratified squamous epithelium pushing it toward proliferation and leading to esophageal squamous cell carcinoma (ESCC).

Using a mouse constitutive *Grhl3* knockout model, the authors assessed the function of GRHL3 first in the developing esophagus. Cellular proliferation was enhanced in knockout esophageal epithelium leading to a thickening of embryonic esophageal epithelium. These results indicate that in the developing esophagus, GRHL3 loss disrupts the proliferation/differentiation balance. To study GRHL3 function in the mature, adult esophageal epithelium, the team developed a conditional *Grhl3* knockout model with deletion controlled by ED-L2-Cre. Like the embryonic tissue, GRHL3 disruption in the mature tissue compromised the proliferation/differentiation balance, skewing the tissue toward a hyperproliferative state. To assess the potential for GRHL3 to act as a tumor suppressor in the esophagus, the authors used the chemical carcinogen 4-nitroquinolene-1 oxide (4-NQO) to induce ESCC in wild-type and *Grhl3* conditional knockout mice. Strikingly, *Grhl3* conditional knockout mice treated with 4-NQO quickly lost substantial amounts of weight. Large, occluding SCCs were present by 26 weeks, compared with wild-type mice, which developed smaller, nonoccluding SCCs. Histologic scoring identified a predominance of SCC, papillomas, and dysplasia in tumors from *Grhl3*-cKO mice, whereas wild-type tissue predominantly consisted of hyperplasia. These observations show that GRHL3 functions as a tumor suppressor in the esophageal epithelium and that its deletion promotes ESCC by increasing the cancer susceptibility of deficient cells.

To elucidate the molecular mechanism through which GRHL3 suppresses cancer, Georgy et al[7] took the logical approach of identifying genes with altered transcription in the absence of GRHL3. They applied rigorous criteria to their transcriptome analysis to define bona fide GRHL3-mediated pathways disrupted in esophageal cancer. By comparing expression profiles among baseline tissues and tumor tissues from wild-type mice and conditional knockout mice, the investigators identified the HOP homeobox (Hopx) protein as significantly downregulated in both groups. HOPX had previously been proposed as a tumor suppressor in ESCC[8] and in head and neck SCC.[9] To pin down GRHL3 as a direct regulator of HOPX, the group performed chromatin immunoprecipitation and identified GRHL3 occupancy at the *Hopx* gene in esophageal cells. Their transcriptome and pathway analysis further revealed dysregulated WNT signaling in GRHL3-deficient tumors, an interesting finding given that HOPX deletion expands WNT signaling in cardiomyocytes.[10] Using chromatin immunoprecipitation, HOPX was shown to occupy the *Wnt2* and *Wnt9a* genes, both upregulated in GRHL3-deficient tumors, and the *Met* gene, a WNT pathway transcriptional target. Consistent with WNT activation, a marked increase in nuclear β-catenin occurred in tumors from *Grhl3* conditional knockout mice. Interestingly, WNT2 and WNT9A are also dysregulated in head and neck SCC.[11] A crucial component of this study was substantiating their observations from mice in human esophageal squamous cell tumors. Pairwise comparisons of more than 50 tumor and matched normal samples revealed a highly significant correlation between *GRHL3* and *HOPX*, with both being downregulated in tumor tissue. Accordingly, *WNT2*, *WNT9A*, and *MET* were consistently upregulated in tumors. Consistent with the transcriptome analysis, immunohistochemical analysis of patient tumors showed GRHL3 and HOPX protein loss in tumors and induction of WNT9A. A dramatic increase in nuclear β-catenin staining confirmed hyperactivation of WNT signaling in GRHL3-deficient human ESCC tissue.

Taken together, Georgy et al[7] identify a GRHL3/HOPX/WNT axis active in esophageal squamous cells that regulates the balance between cellular proliferation and differentiation. When disrupted, cellular proliferation becomes unrestrained, promoting tumorigenesis. The study mechanistically links GRHL3, HOPX, and WNT, each of which had previously been implicated independently in SCC.[8,9,11] There is an urgent need for innovative, efficacious ESCC therapies, and this study supports WNT inhibition as a

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promising approach to treat esophageal tumors with reduced GRHL3 protein. A next step toward this goal would include testing Food and Drug Administration–approved WNT inhibiting drugs in *Grhl3* conditional knockout mice treated with 4-NQO. Finally, acknowledging that WNT-based therapies are challenging because of the essential role of WNT in stem cells, the GRHL3/HOPX/WNT axis identified here could be further explored to uncover a more refined set of ESCC-specific targets for therapeutic interventions.

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Conflicts of interest
The authors disclose no conflicts.

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