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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

SOX15 Governs Transcription in Human Stratified Epithelia and a Subset of Esophageal Adenocarcinomas

Rita Sulahian,[1,2] Justina Chen,[1] Zoltan Arany,[3] Unmesh Jadhav,[1,2] Shouyong Peng,[1] Anil K. Rustgi,[4] Adam J. Bass,[1,2] Amitabh Srivastava,[5] Jason L. Hornick,[5] and Ramesh A. Shivdasani[1,2]

[1]*Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts;* [2]*Department of Medicine, Harvard Medical School, Harvard University, Boston, Massachusetts;* [3]*Cardiovascular Institute and* [4]*Division of Gastroenterology, Departments of Medicine and Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;* [5]*Department of Pathology, Brigham & Women's Hospital, and Department of Pathology, Harvard Medical School, Harvard University, Boston, Massachusetts*

SUMMARY

This study identifies SOX15 as a direct transcriptional regulator of a substantial fraction of cell type-specific genes in stratified epithelial cells. SOX15 expression is attenuated in intestinal metaplasia (Barrett's esophagus) but is active in many esophageal adenocarcinomas.

BACKGROUND & AIMS: Intestinal metaplasia (Barrett's esophagus, BE) is the principal risk factor for esophageal adenocarcinoma (EAC). Study of the basis for BE has centered on intestinal factors, but loss of esophageal identity likely also reflects the absence of key squamous-cell factors. As few determinants of stratified epithelial cell-specific gene expression have been characterized, identifying the necessary transcription factors is important.

METHODS: We tested regional expression of mRNAs for all putative DNA-binding proteins in the mouse digestive tract and verified the esophagus-specific factors in human tissues and cell lines. Integration of diverse data defined a human squamous esophagus-specific transcriptome. We used chromatin immunoprecipitation with high-throughput sequencing (ChIP-seq) to locate transcription factor binding sites, computational approaches to profile the transcripts in cancer data sets, and immunohistochemistry to reveal protein expression.

RESULTS: The transcription factor Sex-determining region Y-box 15 (*SOX15*) is restricted to esophageal and other murine and human stratified epithelia. *SOX15* mRNA levels are attenuated in BE, and its depletion in human esophageal cells reduces esophageal transcripts significantly and specifically. SOX15 binding is highly enriched near esophagus-expressed genes, indicating direct transcriptional control. *SOX15* and hundreds of genes coexpressed in squamous cells are reactivated in up to 30% of EAC specimens. Genes normally confined to the esophagus or intestine appear in different cells within the same malignant glands.

CONCLUSIONS: These data identify a novel transcriptional regulator of stratified epithelial cells and a subtype of EAC with bi-lineage gene expression. Broad activation of squamous-cell genes may shed light on whether EACs arise in the native stratified epithelium or in ectopic columnar cells. *(Cell Mol Gastroenterol Hepatol 2015;1:598–609; http://dx.doi.org/10.1016/j.jcmgh.2015.07.009)*

Keywords: Barrett's Esophagus; Esophageal Gene Regulation; Esophageal Transcriptome; SOX15 Cistrome.

Intestinal metaplasia of the esophagus (Barrett's esophagus, BE) is a common, chronic condition in which an epithelium containing intestinal goblet and other columnar cells replaces the native stratified squamous mucosa.[1] BE results from chronic acid and bile reflux. Over time, the metaplastic tissue may become dysplastic, and it progresses to invasive cancer in three to five cases per 1000 person-years.[2] Esophageal adenocarcinoma (EAC) arises principally in the setting of BE, and the incidence of this cancer in the West increased about eightfold between 1970 and 2010, with about 18,000 new U.S. cases and 15,000 deaths expected in 2015 (http://seer.cancer.gov).

Investigation into the mechanisms of BE has centered largely on determinants of intestinal identity,[3] particularly the intestine-restricted transcription factors (TFs) Caudal type homeobox 1 (CDX1) and CDX2, which specify the embryonic intestine.[4] Forced expression of CDX2 or CDX1 in the mouse stomach induces ectopic intestinal differentiation,[5,6] and both factors are implicated in activating intestinal genes in BE,[7,8] though forced CDX2 expression in the

Abbreviations used in this paper: **BE, Barrett's esophagus; CDX1/2, caudal type homeobox 1/2; ChIP, chromatin immunoprecipitation; ChIP-seq, chromatin immunoprecipitation with high-throughput sequencing; EAC, esophageal adenocarcinoma; G-E, gastroesophageal; KRT5, keratin 5, type II; KRT6A, keratin 6A, type II; PAX9, paired box 9; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; shRNA, small hairpin RNA; SIM2, single-minded family bHLH transcription factor 2; SOX2, 15, sex-determining region Y-box 2, -box 15; TCGA, The Cancer Genome Atlas; TF, transcription factor; TP63, tumor protein P63; TRIM29, tripartite motif containing 29.**

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mouse esophagus does not induce BE per se.[9] Loss of esophagus-specific transcripts and of stratified squamous morphology probably reflects parallel loss of transcriptional determinants of the native epithelium, which are largely unknown. Tumor protein P63 (TP63) regulates differentiation of all stratified epithelia, such as those in the esophagus and skin,[10,11] acting in part through another transcription factor, basonuclin 1 (BNC1).[12] Sex-determining region Y-box 2 (SOX2) controls esophageal differentiation in embryos[13] and growth of adult progenitor cells,[14,15] an activity in which Kruppel-like factor 4 (KLF4) and KLF5 also may participate.[16] Forkhead box A2 (FOXA2) is expressed in embryonic but not in adult esophageal cells.[17] We sought to identify other tissue-restricted TFs that might control the characteristic stratified epithelium.

Among all putative DNA-binding proteins, we searched first for those with esophagus-restricted expression among digestive epithelia and then for factors with attenuated expression in BE. We identified sex-determining region Y-box 15 (SOX15) as such a TF and we show that it directly controls transcription of a large fraction of human esophagus-expressed genes. *SOX15* is absent from most EACs, but up to 30% of cases retain expression of *SOX15* and its target genes, coexpressing representative intestinal and squamous-specific genes within the same tissue. Together, these data identify a novel regulator of stratified epithelial genes and a subtype of EAC with bi-lineage gene expression.

Materials and Methods

Tissue Preparation and Transcription Factor Expression Screen

We isolated epithelial sheets from the esophagus, gastric corpus-antrum, and duodenum of 1-month old CD1 and C57BL/6 mice. Before peeling the mucosa using fine forceps, the esophagus was treated with 0.1% collagenase-dispase (cat. no. 11097113001; Roche Applied Science, Indianapolis, IN) in phosphate-buffered saline (PBS) for 15 minutes at 37°C, whereas stomach and duodenum were incubated in 1 mM ethylenediaminetetraacetic acid (EDTA) in PBS at 37°C. To determine the relative transcript levels (Figure 1*A–C*), we used quantitative reverse-transcription

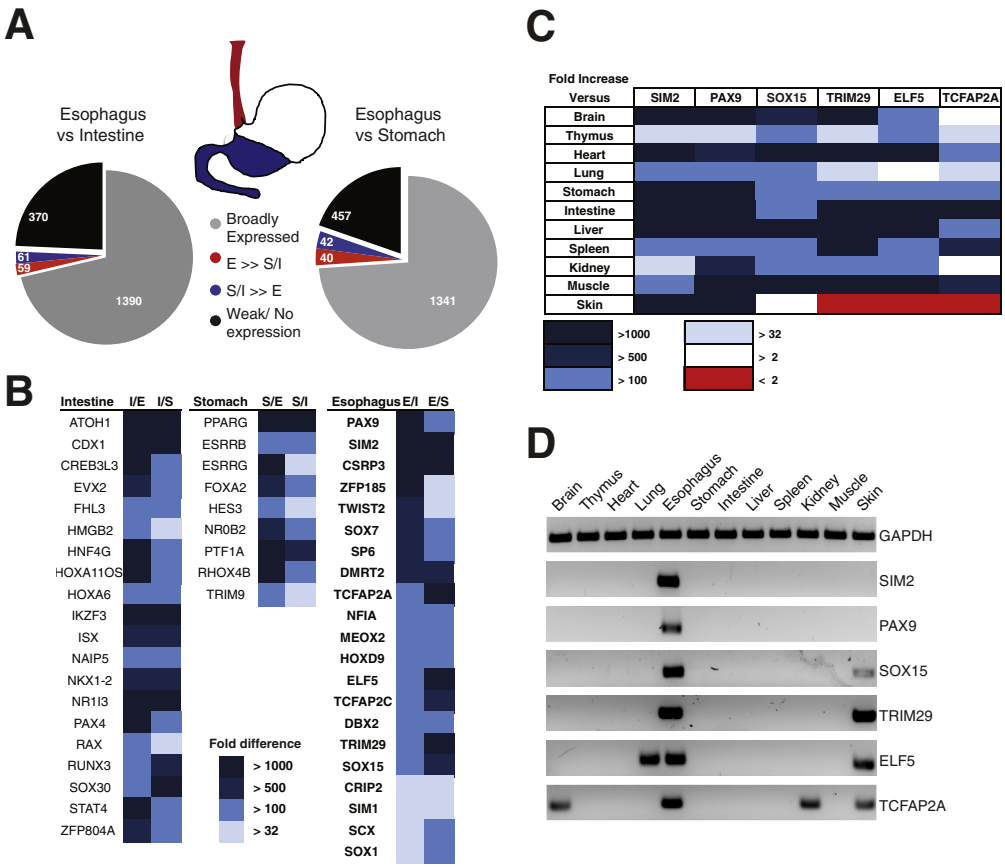

Figure 1. Differential transcription factor (TF) expression in the normal mouse gut and other tissues. (*A*) Distribution of all TFs in wild-type mouse digestive epithelia, as revealed in a quantitative reverse-transcription polymerase chain reaction (qRT-PCR) screen. Expression of 1880 TF mRNAs was assessed in epithelial cell isolates from adult CD1 mouse esophagus (*red*), stomach and intestine (*blue*). (*B*) TFs restricted to intestinal (I), stomach (S) or esophageal (E) epithelium, with the fold-excess over other tissues represented in shades of blue. (*C*) Relative expression of *Sim2*, *Pax9*, *Sox15*, *Trim29*, *Elf5*, and *Tcfap2a* mRNAs in mouse tissues. The fold-excess values are represented in shades of color as indicated in the key. (*D*) Products of qRT-PCR for the six most highly esophagus-specific TF mRNAs in 12 adult mouse organs, showing selective expression in the esophagus and of some factors in the skin.