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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Transcriptional Regulation by ATOH1 and its Target SPDEF in the Intestine

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SUMMARY

The transcription factor ATOH1 is critical for Notch-mediated differentiation and maturation of intestinal secretory cells. Here we identify direct targets of ATOH1 in mouse small intestine and colon.

BACKGROUND & AIMS: The transcription factor atonal homolog 1 (ATOH1) controls the fate of intestinal progenitors downstream of the Notch signaling pathway. Intestinal progenitors that escape Notch activation express high levels of ATOH1 and commit to a secretory lineage fate, implicating ATOH1 as a gatekeeper for differentiation of intestinal epithelial cells. Although some transcription factors downstream of ATOH1, such as SPDEF, have been identified to specify differentiation and maturation of specific cell types, the bona fide transcriptional targets of ATOH1 still largely are unknown. Here, we aimed to identify ATOH1 targets and to identify transcription factors that are likely to co-regulate gene expression with ATOH1.

METHODS: We used a combination of chromatin immunoprecipitation and messenger RNA–based high-throughput sequencing (ChIP-seq and RNA-seq), together with cell sorting and transgenic mice, to identify direct targets of ATOH1, and establish the epistatic relationship between ATOH1 and SPDEF.

RESULTS: By using unbiased genome-wide approaches, we identified more than 700 genes as ATOH1 transcriptional targets in adult small intestine and colon. Ontology analysis indicated that ATOH1 directly regulates genes involved in specification and function of secretory cells. De novo motif analysis of ATOH1 targets identified SPDEF as a putative transcriptional co-regulator of ATOH1. Functional epistasis experiments in transgenic mice show that SPDEF amplifies ATOH1-dependent transcription but cannot independently initiate transcription of ATOH1 target genes.

CONCLUSIONS: This study unveils the direct targets of ATOH1 in the adult intestines and illuminates the transcriptional events that initiate the specification and function of intestinal secretory lineages. *(Cell Mol Gastroenterol Hepatol 2017;3:51–71; http://dx.doi.org/10.1016/j.jcmgh.2016.10.001)*

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See editorial on page 2.

The adult intestinal epithelium proliferates rapidly with average cellular lifespans of approximately 5–7 days. To maintain epithelial integrity and perform its major function of nutrient digestion and absorption, intestinal stem cells (ISCs) located at the base of crypts of Lieberkühn must self-renew and produce transit-amplifying cells, which subsequently differentiate into 1 of 2 cell classes: absorptive lineage cells, including enterocytes and colonocytes; and secretory lineage cells, including mucus-secreting goblet cells, hormone-secreting enteroendocrine cells, and antimicrobial peptide-secreting Paneth cells.[1–3] Under physiological conditions, signaling pathways, such as Notch and Wnt, modulate homeostasis and differentiation of the intestinal epithelium, directing ISCs/progenitors toward either the absorptive or secretory fate by controlling the expression of a downstream transcriptional network.[4,5] Dysregulated ISC proliferation or aberrant differentiation may cause gastrointestinal diseases, such as inflammatory bowel disease and intestinal cancer.[5,6]

Canonical Notch signaling relies on direct cell–cell contact and plays an important role in modulating homeostasis and differentiation of the intestinal epithelium. In the intestines, Notch signaling controls the fate of ISCs/progenitors by regulating the expression of the basic

Abbreviations used in this paper: ATOH1, atonal homolog 1; ChIP, chromatin immunoprecipitation; ChIP-seq, chromatin immunoprecipitation sequencing; CRC, colorectal cancer; DBZ, dibenzazepine; FACS, fluorescence-activated cell sorting; FDR, false-discovery rate; Gfi1, growth factor independent 1; GFP, green fluorescent protein; GO, gene ontology; ISC, intestinal stem cell; mRNA, messenger RNA; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; QES, Q-enrichment-score; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; Spdef, SAM pointed domain containing ETS transcription factor; TSS, transcription start site.

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helix-loop-helix transcription factor atonal homolog 1 (ATOH1).[5] Previous studies have suggested that ATOH1 is required for the differentiation of all secretory cells.[7] Germline *Atoh1* deletion causes mice to die shortly after birth and fail to form any secretory cells without affecting enterocytes.[7] Consistent with these observations, conditional deletion of *Atoh1* in the adult intestinal epithelium results in the loss of all secretory cells.[8] In contrast, overexpression of ATOH1 directs progenitor cells to the secretory cell fate in the embryonic intestine.[9] Previous studies have indicated that pharmacologic inhibition of Notch signaling using γ-secretase inhibitors or specific antibodies blocking the Notch receptors results in loss of proliferative progenitor cells and secretory cell hyperplasia.[10–12] However, *Atoh1*-deficient intestines fail to respond to Notch inhibition, indicating that the primary role of Notch is to regulate the expression of *Atoh1*, and in doing so control secretory vs absorptive cells fate.[13–15] Consistent with the concept, a recent study suggested that ATOH1 controls Notch-medicated lateral inhibition in the adult intestinal epithelium.[16] These results indicate that ATOH1 is a critical gatekeeper for the program of Notch-mediated differentiation and cell fate determination of intestinal epithelial cells. Although previous studies have suggested that some transcription factors, such as SAM pointed domain containing ETS transcription factor (*Spdef*) and growth factor independent 1 (*Gfi1*), are downstream of ATOH1 and are important for differentiation of specific secretory cell types,[17–19] the bona fide targets of endogenous ATOH1 at the genome-wide level in the adult intestine still largely are unknown.

To better understand the molecular functions of ATOH1 in vivo, we used a combination of chromatin immunoprecipitation (ChIP) and RNA-based, high-throughput sequencing techniques to identify direct transcriptional targets of ATOH1 in ileal and colonic crypts. In addition, our data unveiled a novel molecular mechanism whereby SPDEF functions as a transcriptional co-regulator of ATOH1, amplifying ATOH1-dependent transcription of a subset of secretory genes. This study provides novel insight toward understanding cell fate decisions within the intestines.

Materials and Methods

Animals

VilCre^{ERT2}; Fabp1^{Cre}; Atoh1$^{fl/fl}$; Rosa26 $^{LSL\text{-}rtta\text{-}ires\text{-}EGFP}$; TRE-Spdef; Spdef null; Atoh1$^{GFP/GFP}$; and Atoh1$^{Flag/Flag}$ mice have been described previously.[8,18,20–24] To achieve deletion of *Atoh1* from intestinal epithelium, Atoh1$^{fl/fl}$; VilCre^{ERT2} mice and littermate controls were given an intraperitoneal injection of 1 mg/mouse tamoxifen (Sigma, St. Louis, MO) dissolved in corn oil for 3 consecutive days. Animals were killed 5 days after the first injection. To achieve SPDEF induction, Fabp1^{Cre}; Atoh1$^{fl/fl}$; Rosa26 $^{LSL\text{-}rtta\text{-}ires\text{-}EGFP}$; TRE-Spdef mice, and littermate controls were given 2 mg/mL tetracycline in water for 5 consecutive days. To achieve Notch inhibition, mice were treated either with vehicle or GSI-20 (also called dibenzazepine [DBZ]; EMD–Calbiochem, Darmstadt, Germany) at 15 μmol/L/kg once a day for 5 days. All mouse studies were approved by the Institutional Animal Care and Use Committee.

Crypt Isolation

Intestinal crypts were prepared as previously described.[25] Entire colons and 6–7 cm distal small intestine were dissected out and flushed with ice-cold phosphate-buffered saline (PBS) with 5 mmol/L phenylmethylsulfonyl fluoride. Intestines were opened lengthwise and cut into 1-cm pieces. Tissues were incubated with shaking buffer (25 mmol/L EDTA, protease inhibitor cocktail; Calbiochem) at 4°C for 30 minutes by gentle shaking. Shaking buffer was replaced by ice-cold Ca^{2+}/Mg^{2+}-free Dulbecco's PBS followed by vigorous shaking for approximately 8–10 minutes to generate disassociated crypts. For the colon, it takes 15 minutes to disassociate crypts. Intestinal crypts were isolated by filtering through a 70-μm cell strainer (BD Falcon) for small intestinal crypts and a 100-μm cell strainer (BD Falcon, Tewksbury, MA) for colonic crypts, and then spun down at 150*g* for 10 minutes.

Cell Culture

Human colorectal cancer cell line HCT was grown in RPMI1640 (10-040-CV; Corning, New York, NY) supplemented with 10% fetal bovine serum (S1200-500; BioExpress, Kaysville, UT), penicillin, and streptomycin (17-602E; Lonza, Basel, Switzerland).

Plasmids and DNA Transfection

Expression plasmid of ATOH1-GFP was a gift from Dr Tiemo Klisch (Baylor College of Medicine).[20] HCT116 green fluorescent protein (GFP) cells were transfected by using Lipofectamine 2000 (11668-019; Invitrogen, Waltham, MA) following the manufacturer's instructions.

Chromatin Immunoprecipitation

Crypts and transfected cells were used in chromatin immunoprecipitation (ChIP) experiments with antibodies against GFP (NB600-303; Novus, Littleton, CO), Flag M2 (F1804; Sigma), H3k27Ac (ab4729; Abcam, Cambridge, MA), or H3K27me3 (ab6002; Abcam). For each ChIP sample, 2–3 μg of antibodies were used to bind to 10 μL Protein G Dynabeads (100-03D; Invitrogen) following the manufacturer's instructions. Samples from either crypts or 5–10 $\times$ 10^6 HCT116 cells transfected with ATOH1-GFP were cross-linked in 1% formaldehyde (15710; Electron Microscopy Sciences, Hatfield, PA) in cross-linking buffer (50 mmol/L HEPES pH 8.0, 1 mmol/L EDTA pH 8.0, 1 mmol/L ethylene glycol-bis[β-aminoethyl ether]-*N,N,N′,N′*-tetraacetic acid pH 8.0, 100 mmol/L NaCl, RPMI1640) at room temperature for 30 minutes and then quenched by adding glycine to a final concentration of 135 mmol/L on ice for 5 minutes. Cross-linked cells were washed twice with ice-cold PBS and stored at -80°C before sonication. Chromatin was sheared to 300- to 1000-bp fragments in 1 mL ice-cold sonication buffer (10 mmol/L Tris-HCl pH 8.0, 1 mmol/L EDTA pH 8.0, 1 mmol/L ethylene glycol-bis[β-aminoethyl ether]-*N,N,N′,N′*-tetraacetic acid pH 8.0, supplemented with a protease inhibitor cocktail; 539134; Calbiochem), using a 250D Sonifier Ultrasonic Processor Cell Disruptor (Branson, Danbury, CT) with a one-eighth inch microtip (50% power

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