XBP1 Controls Maturation of Gastric Zymogenic Cells by Induction of MIST1 and Expansion of the Rough Endoplasmic Reticulum

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BACKGROUND & AIMS: The transition of gastric epithelial mucous neck cells (NCs) to digestive enzyme–secreting zymogenic cells (ZCs) involves an increase in rough endoplasmic reticulum (ER) and formation of many large secretory vesicles. The transcription factor MIST1 is required for granulogenesis of ZCs. The transcription factor XBP1 binds the *Mist1* promoter and induces its expression in vitro and expands the ER in other cell types. We investigated whether XBP1 activates *Mist1* to regulate ZC differentiation. **METHODS:** *Xbp1* was inducibly deleted in mice using a tamoxifen/Cre-loxP system; effects on ZC size and structure (ER and granule formation) and gastric differentiation were studied and quantified for up to 13 months after deletion using morphologic, immunofluorescence, quantitative reverse-transcriptase polymerase chain reaction, and immunoblot analyses. Interactions between XBP1 and the *Mist1* promoter were studied by chromatin immunoprecipitation from mouse stomach and in XBP1-transfected gastric cell lines. **RESULTS:** Tamoxifen-induced deletion of *Xbp1* (*Xbp1*Δ) did not affect survival of ZCs but prevented formation of their structure. *Xbp1*Δ ZCs shrank 4-fold, compared with those of wild-type mice, with granulogenesis and cell shape abnormalities and disrupted rough ER. XBP1 was required and sufficient for transcriptional activation of *MIST1*. ZCs that developed in the absence of XBP1 induced ZC markers (intrinsic factor, pepsinogen C) but showed abnormal retention of progenitor NC markers. **CONCLUSIONS: XBP1 controls the transcriptional regulation of ZC structural development; it expands the lamellar rough ER and induces *MIST1* expression to regulate formation of large granules. XBP1 is also required for loss of mucous NC markers as ZCs form.**

Keywords: Maturation; Stem Cell; Cell Structures; Gastric Acid Secretion.

BASIC–ALIMENTARY TRACT

The corpus of the mouse stomach is an excellent tissue for studying developmentally regulated transcription factors (TFs) in generation of secretory cell architecture, because the epithelium turns over continuously throughout adult life. In addition, the gastric epithelial stem cell gives rise to several diverse secretory lineages. Zymogenic cells (ZCs), for example, reside at the base of gastric epithelial glands and develop after a prolonged (~2 weeks) phase as progenitor cells, known as mucous neck cells (NCs), which in turn differentiate from the gastric epithelial stem cell.[1,2] Thus, for the ZC lineage, distance from the progenitor zone corresponds to the differentiation stage.

The TF MIST1 is involved in ZC differentiation. In *Mist1*$^{-/-}$ mice, ZCs delay turning off progenitor markers as they arise from NCs, although fully differentiated ZCs eventually form in normal numbers. However, all *Mist1*$^{-/-}$ ZCs are structurally defective with deficient apical cytoplasms and small secretory vesicles, although they show normal deposition of elaborate, lamellar rough endoplasmic reticulum (rER).[3,4] The function of MIST1 as a secretory cell-specific structure-inducing TF is highly conserved: even in flies, the MIST1 orthologue DIMM mediates granule structure of peptide-secreting cells without affecting survival.[5,6]

Despite the complex and interesting developmental patterning in the gastric epithelium, little is known about the underlying transcriptional and molecular mechanisms. Some progress has been made in understanding morphogens in gastric patterning. For example, epidermal growth factor receptor ligands transforming growth factor α/epidermal growth factor/amphiregulin drive increased surface cell growth[7,8]; the Hedgehog pathway seems to be required for inhibiting surface cell growth and promoting NC transition into ZCs[9,10]; and various cytokines, such as interleukin-1β and interleukin-11, and in general the nuclear factor κB signaling pathway, seem to be key in regulating growth and multiple differentiation pathways.[9,11]

Other than MIST1, only a handful of other TFs play a known role in differentiation of adult corpus epithelial

Abbreviations used in this paper: ER, endoplasmic reticulum; NC, mucous neck cell; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; rER, rough endoplasmic reticulum; SPEM, spasmolytic polypeptide expressing metaplasia; TF, transcription factor; TM, tamoxifen; ZC, zymogenic cell.

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lineages: FOXQ1, which regulates granule maturation in mucus-secreting surface (aka pit/foveolar) cells[12]; NGN3 and MASH1,[13,14] which regulate development of hormone-secreting endocrine cells; and KLF4, which apparently regulates differentiation of multiple secretory lineages.[15]

XBP1 is a TF traditionally viewed as a key regulator of the unfolded protein response during endoplasmic reticulum (ER) stress. XBP1 messenger RNA is spliced and thereby activated by IRE1, which governs part of the unfolded protein response.[16] XBP1 has also been described as a developmentally regulated TF that induces ER expansion and may be required for differentiation of dedicated secretory cells, such as antibody-secreting plasma cells and intestinal Paneth cells.[17–19] It is unclear, however, whether XBP1 is required for cell survival and fate determination like other developmentally regulated TFs or whether it plays a special role in establishing differentiated cell function rather than cell identity.[20,21] Interestingly, an in vitro screen recently identified MIST1 as a transcriptional target of XBP1 in myoblasts, plasma cells, and pancreatic β cells.[22] It is not clear whether XBP1 is required for induction of MIST1 or whether XBP1 targets MIST1 in vivo.

Here, we examine the role of XBP1 in gastric epithelial differentiation. Using inducible deletion with tamoxifen-Cre-loxP, we show that XBP1 is required for nearly the entire structural development of ZCs, including elaboration of rER and formation of large secretory granules. XBP1 induces MIST1 in gastric epithelial cell lines and, in mice, is required for induction of *Mist1* expression in ZCs. Interestingly, ZCs arising in the absence of XBP1 still induce normal ZC differentiation markers such as gastric intrinsic factor, but they cannot extinguish expression of progenitor NC markers; in other words, they never terminally differentiate. Thus, XBP1 is absolutely required for structural differentiation and maturation of ZCs but is dispensable for survival and initial induction of the ZC fate. The results show for the first time that XBP1 is the principal governor of ZC structural maturation, plays a role in shutting off progenitor features, and is required for induction of *Mist1* in vivo.

Materials and Methods

Mice

All experiments involving animals were performed according to protocols approved by the Washington University School of Medicine Animal Studies Committee. Floxed *Xbp1,* CAGGCreER™ transgenic mice, and germline $Xbp1^{-/-}$ mice with liver *Xbp1* transgene ($Xbp1^{-/-}$; Liv^{XBP1}) were generated as described previously.[17,23–26] $Xbp1^{flox/flox}$ mice were crossed with CAGGCreER™ transgenic mice, and then CAGGCreER™ tg; $Xbp1^{flox/+}$ mice were crossed with $Xbp1^{flox/+}$ to generate CAGGCreER™ tg; $Xbp1^{flox/flox}$ as well as a variety of control mice. Tamoxifen (0.75 mg/20 g body wt; Sigma, St Louis, MO) was injected intraperitoneally for 7 consecutive days to induce gene deletion. Mice were killed 7 days, 14 days, 28 days, 5 months, 7 months, and 13 months after the first injection of tamoxifen.

Cell Imaging

Transmission electron microscopy,[4] terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling, bromodeoxyuridine,[27] and other immunofluorescence studies were as described[3,4,28]; goat anti-calregulin (Santa Cruz Biotechnology, Santa Cruz, CA) was diluted 1:200. Immunofluorescent quantification to determine cytoplasmic fluorescence intensity was performed using ImageJ software with methods described previously[3]; however, for the current study, the mean fluorescent intensity for each cell was normalized to the maximum mean fluorescent intensity for that channel in each unit to generate the percent maximal mean fluorescent intensity. In $Xbp1^{\Delta}$ mice, 351 cells in 14 units from 2 mice were quantified; in controls, 225 cells in 9 units from 5 mice were quantified.

Stomach Chromatin Immunoprecipitation

Mouse stomach chromatin immunoprecipitation was performed following Wells and Farnham.[29] One stomach was dissected for one experiment, and the experiment was repeated with another mouse showing similar results. A total of 10 μL of rabbit anti-XBP1 antibody (Santa Cruz Biotechnology) with protein A/G plus agarose (Santa Cruz Biotechnology) were added to the tissue lysate for immunoprecipitation. A consensus XBP1 binding motif in the *Mist1* promoter was described previously,[22] and using ECR browser (http://ecrbrowser.dcode.org), we noted this site was conserved from human to mouse to opossum (not shown). Primers spanning the putative XBP1 binding site were designed with Primer3 (http://frodo.wi.mit.edu/primer3/) (Supplementary Table 1). Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) with these primers was performed to assess the quantity of genomic sequences immunoprecipitated by anti-XBP1 antibody, as well as a 1:10 dilution of the cell extract before immunoprecipitation, and a control genomic region lacking consensus XBP1 binding sites.

Cell Line and Transient Transfection

AGS cells (a human gastric carcinoma cell line; American Type Culture Collection, Manassas, VA) were grown and transfected by Nucleofection as described[3] with 3 μg hXbp1(s) and 2 μg pmaxGFP (Lonza, Walkersville, MD). qRT-PCR analysis was as described.[30]