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XBP1

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Gene Symbol: ***Xbp1***

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1. General Information

X-box binding protein 1 (XBP1) is member of the cAMP response element binding/ Activating transcription factor (CREB/ATF) family of basic region leucine zipper (bZIP) transcription factors (4), and it was initially discovered through analysis of proteins that bound to an X-box motif in the major histocompatibility complex (MHC) class II, *downregulated in adenoma* (*DRA*) gene in humans and the MHC class II antigen A, alpha (*Aa*) gene in mice (20). *Xbp1* is the mammalian homologue to the yeast gene *Hac1*, an important mediator of the unfolded protein response (UPR).

XBP1 transcriptional activity is regulated by inositol-requiring enzyme 1 (IRE1), the transducer of one of three signaling cascades activated by the unfolded protein response.

Under non-stressed conditions, IRE1 is associated with glucose responsive protein 78 (GRP78/BiP) in an inactive state. When unfolded proteins accumulate in the ER lumen they can sequester BiP, or bind directly with IRE1 monomers (7) resulting in its oligomerization and trans-autophosphorylation of its kinase domain. Activation of IRE1 results in endoribonuclease (RNase) activity (25, 31) that splices a 26 base pair fragment out of the *Xbp1* mRNA from base pairs 531 to 556 (32). While the unspliced *Xbp1* (*Xbp1u*) generates a protein that is 261 amino acids in size (3, 26, 32), the splicing of *Xbp1u* mRNA causes a frameshift and generates a 376 amino acid XBP1s that juxtaposes the bZIP DNA binding domain with a potent transactivation domain in the shifted and extended C-terminus. This alteration confers stability and transcriptional activation ability to XBP1s (32). Both *Xbp1u* and *Xbp1s* are translated, but XBP1u is believed to be highly labile and is rapidly degraded. Interestingly, recent evidence suggests XBP1u can repress the transcriptional activity of the UPR (28, 33, 34).

The transcriptional targets of XBP1 vary based on tissue type (1, 16). Studies in mouse embryonic fibroblasts (MEFs) lacking *Xbp1* revealed that two XBP1 targets are UPR regulators, the ER chaperones DnaJ (*Hsp40*) homolog, subfamily B, member 9 (*DnaJB9; ERdj4; MDG1*) and protein kinase inhibitor of 58 kDa ($p58^{IPK}$; *DnaJC3*) (17). ChIP-on-chip analysis aimed at determining transcriptional networks governed by XBP1 revealed that XBP1 constitutively binds to genes involved in ER homeostasis, including PRKR-like endoplasmic reticulum kinase (PERK; *Eif2ak3*)

and GRP78/BiP (1).

XBP1 is required for plasma cell differentiation and consequent Ig production (23) and is a transcriptional activator of interleukin-6 (*Il6*) (12), indicating that XBP1 is crucial for adaptive immunity. XBP1 has also been implicated in adipocyte differentiation and hepatic lipid metabolism, since XBP1 regulates expression of the lipogenic genes acetyl Co-A carboxylase (*ACC2*), stearoyl Co-A reductase (*SCD1*) and diacylglycerol acetyltransferase 2 (*DGAT2*) by binding to their promoter regions (18). Recently, XBP1 has been implicated in bone morphogenetic-2-induced osteoblast differentiation through its transcriptional regulation of *osterix*, a gene required for bone development (29). XBP1 is also required for the proper differentiation of zymogenic chief cells. Zymogenic chief cells are similar to pancreatic acinar cells in that they are highly secretory and require expression of Mist1 for proper differentiation (10).

Complete loss of *Xbp1* through targeted ablation in mice (*Xbp1*$^{-/-}$) results in embryonic lethality by embryonic day (E) 14.5. Embryonic lethality was attributed to the development of hypoplastic fetal livers resulting in reduced hematopoiesis and anemia (22), and was rescued by maintaining XBP1 expression in the liver (*Xbp1*$^{-/-}$*; Liver*^{Xbp1}) (16). Most *Xbp1*$^{-/-}$*; Liver*^{Xbp1} exhibited perinatal lethality, with symptoms of poor nutritional status, growth retardation and hypoglycemia. Interestingly, *Xbp1*$^{-/-}$*; Liver*^{Xbp1}mice display a severe exocrine pancreas phenotype (16) that implicates XBP1 in acinar cell development and function.

2. XBP1 and the Exocrine Pancreas

Pancreatic acinar cells are highly secretory in nature and have a high rate of protein production. Since most proteins produced in acinar cells are secreted, there is a high demand for protein folding in the ER when secretion is stimulated, requiring proper management of protein synthesis and folding machinery. This requirement suggests that the pancreatic acinar cell relies on the UPR (and thus the IRE1/XBP1 pathway) for efficient function. Generation of XBP1-reporter transgenic mouse lines has confirmed that XBP1 is constitutively active in the pancreas (13, 27).

Xbp1 expression is first detected in the mouse pancreas at E12.5, coinciding with the second wave of differentiation. Its expression increases to a maximum by E14.5, only to decrease to lower levels by E18.5 (5), suggesting a developmental role for XBP1.

The Glimcher group at Harvard generated knockout mouse lines to examine the role of XBP1 in various tissues (16). A global XBP1 knockout (*Xbp1*$^{-/-}$) was embryonic lethal where no viable *Xbp1*$^{-/-}$ embryos were obtained after E14.5. Embryonic lethality was rescued in the *Xbp1*$^{-/-}$ mouse line when XBP1 was re-expressed in the liver (*Xbp1*$^{-/-}$*; Liver*^{Xbp1}). Gross morphological analysis of *Xbp1*$^{-/-}$*;Liv*^{Xbp1} pancreata revealed a 90% decrease in pancreas size when compared to wild type (WT) littermates, where pancreatic tissue consisted of sparsely distributed acini in a loose mesenchymal structure. Transmission electron microscopy (TEM) revealed proper organization of *Xbp1*$^{-/-}$*;Liv*^{Xbp1} acinar cells around a lumen, but with significantly fewer and smaller zymogen granules compared to WT litter mates. Additionally, the endoplasmic reticulum in mutant cells was poorly developed with few cisternae. Pancreatic tissue from these mice also showed drastic reductions in amylase and trypsin accumulation and significant acinar cell apoptosis by E18.5 due to dysregulated ER stress. The importance of the IRE1/XBP1 pathway in pancreatic acinar cells is highlighted by the discovery that loss of the PERK pathway in pancreatic cells does not result in ER stress or apoptosis (11). Interestingly, development of the endocrine pancreas was unaffected in *Xbp1*$^{-/-}$*;Liv*^{Xbp1} mice (16). Thus, this work established a role for XBP1 specifically in development of the

exocrine pancreas.

To more thoroughly determine the role of XBP1 in the pancreatic acinar cell Stephen Konieczny's group crossed *Xbp1*^{flox} (18) mice with *Mist1-Cre*$^{ER/T2}$ mice to generate a line that is null for *Xbp1* only in acinar cells (*Xbp1*$^{\Delta Ex2}$) (8). Analysis of *Xbp1*$^{\Delta Ex2}$ mice 4 weeks after loss of Xbp1 revealed a 60% decrease in expression of amylase and elastase, and increased activity of the UPR. Structurally, *Xbp1*$^{\Delta Ex2}$ acinar cells exhibited decreased accumulation of zymogen granules and decreased amounts of cytoplasm. Further analysis revealed poorly developed and distended ER with disorganized cisternae, and ribosomes were not longer associated with the ER. Additionally, these cells showed increases in ER stress indicators and eventually succumbed to apoptotic cell death, indicating that XBP1 is essential for maintaining both the UPR and acinar cell homeostasis (8).

Transcriptional targets for XBP1 in the pancreas include *Sec61*α, ER degradation enhancer, mannosidase alpha-like (EDEM), protein disulfide isomerase (*PDI*) and the pancreas-specific isoform *PDIp*. Sec61α is required for translocation of newly synthesized proteins across the ER membrane (21), whereas EDEM plays a role in the degradation of misfolded proteins in the ER (9). As the name implies, the PDIs are required for disulfide bond formation and isomerization of newly synthesized proteins (24) within the ER. *PDIp* is exclusively expressed in the exocrine pancreas (6) and is able to bind zymogens, suggesting an important role in zymogen folding (30). XBP1 also directly regulates expression of *Mist1*, an exocrine pancreas developmental gene (1). XBP1 binds to the *Mist1* promoter in the C2C12 myoblast cell line, plasma cells and in MIN6 insulinoma cells, and ectopic XBP1s expression induces MIST1 expression (1). While these studies were not carried out in an acinar cell environment, they indicate that XBP1 can regulate expression of *Mist1*, and that the loss of this interaction in *Xbp1*$^{-/-}$*;Liv*^{Xbp1} mice may account for the observed exocrine pancreas phenotype.

Studies have also aimed at determining XBP1's role during pancreatic injury. Experimental induction of pancreatitis using L-arginine in rats or CCK analogs in isolated rat pancreatic acini induced splicing of *Xbp1* (14, 15). Furthermore, studies from our lab and others have shown that prolonged exposure to ethanol initiated splicing of *Xbp1* in pancreatic tissue (2, 19). While long-term ethanol feeding in mice does not lead to pancreatic deficiency, long-term ethanol feeding of *Xbp1* heterozygote (*Xbp1*$^{+/-}$) mice, which exhibit a 30% decrease in the level of pancreatic XBP1, resulted in areas of acinar cell necrosis, stroma deposition and the presence of tubular complexes. There was also a 25% decrease in the number of zymogen granules per cell and a 30% reduction in amylase expression in *Xbp1*$^{+/-}$ ethanol-fed mice. Other hallmarks of injury in *Xbp1*$^{+/-}$ mice included increased tissue inflammation, increased vacuolization of acinar cells and increased autophagy (19). To date, transcriptional targets of XBP1 during pancreas injury have not been determined. Elucidation of these targets is essential for the complete understanding of the role of XBP1 and its impact in exocrine pancreas pathology.

3. Reagents available for the study of XBP1

a. Antibodies

Multiple XBP1 antibodies are commercially produced. Antibodies that are commonly used in published studies of XBP1 include:

1. Rabbit anti-XBP1 (M-186) from Santa Cruz (cat. # sc-7160). This antibody has been successfully used for Western blotting, and ChIP in various tissues and for immunofluorescence in cultured cells overexpressing XBP1.
2. Rabbit anti-XBP1 from Biolegend (cat. #

619502). This antibody has successfully been used for Western blotting and ChIP in various tissues (18).

b. cDNA clones

Addgene distributes a clone for both unspliced (pFLAG.XBP1u.CMV2) and spliced *Xbp1* (pFLAG.XBP1s.CMV2) conjugated with a FLAG tag (3).

c. siRNA, shRNA

Xbp1-specific siRNA is commercially available through Santa Cruz Biotechnology (sc-38628). Santa Cruz Biotechnology also carries an *Xbp1* shRNA plasmid (sc-38628-SH) and lentiviral particle (sc-38628-V).

d. Genetically modified mice

Mice generated with global loss of *Xbp1* (*Xbp1*$^{-/-}$) exhibit embryonic lethality at approximately E12.5 (23). Reintroduction of *Xbp1* in the liver (*Xbp1*$^{-/-}$*;Liv*^{Xbp1}) rescued embryonic lethality, generating a viable mouse line in which to study the role of *Xbp1* (16). A mouse line harbouring loxP sites in the first and second intron of the *Xbp1* gene (*Xbp1*^{flox}) have also been generated (18), and crossing with *Mist1-Cre*$^{ER/T}$ mice produced an acinar cell specific *Xbp1* null mouse line (*Xbp1*$^{\Delta Ex2}$) (8).

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