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Pharmacological targeting of endoplasmic reticulum stress in pancreatic beta cells

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Abstract:	Diabetes is a disease with pandemic dimensions and no pharmacological treatment prevents disease progression. Dedifferentiation has been proposed to be a driver of beta-cell dysfunction in both type 1 and type 2 diabetes. Regenerative therapies aim to reestablish function in dysfunctional or dedifferentiated beta cells and restore the defective insulin secretion. Unsustainable levels of insulin production with increased demand at disease onset strain the beta-cell secretory machinery leading to endoplasmic reticulum (ER) stress. Unresolved chronic ER stress is a major contributor to beta cell loss of function and identity. Restoring ER homeostasis, enhancing ER-associated degradation of misfolded protein, and boosting chaperoning activity, are emerging therapeutic approaches for diabetes treatment.

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To the Editors of Trends in Pharmacological Sciences

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Please find enclosed our manuscript entitled "**Pharmacological targeting of endoplasmic reticulum stress in pancreatic beta cells**" by Sara Bilekova, Stephan Sachs, and Heiko Lickert, which we submit to *Trends in Pharmacological Sciences* as a review article for revision.

Pancreatic beta-cell dysfunction precedes both type 1 and type 2 diabetes pathogenesis. Endoplasmic reticulum (ER) stress is a major contributor to beta cell dysfunction. The pathways regulating ER homeostasis serve as pharmacological targets for reversing beta-cell dedifferentiation and improving beta-cell function. Here, we review pharmacological modulators of the unfolded protein response, the ER-associated degradation pathway, and chaperoning compounds in beta cells, focusing on the field's development in the recent years.

We would be greatly thankful for a review at *Trends in Pharmacological Sciences* and would like to thank you in advance for your time and consideration.

As reviewers, we would suggest Decio Eizirik (ULB Center for Diabetes Research), who investigates the pathways of type 1 diabetes pathogenesis, focusing among others on the endoplasmic reticulum stress pathway. Next, we would suggest Anil Bhushan (UCSF, Diabetes Center) who studies beta-cell proliferation and regeneration and Lori Sussel (Director, Barabara Davis Center for Childhood Diabetes, Denver) who is an islet and beta-cell expert.

Sincerely,

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Highlights

- Beta-cell dysfunction and dedifferentiation precedes T1D and T2D onset and offers a window of opportunity for early pharmacological intervention.
- Chronic ER stress is the main contributor to beta-cell dysfunction, which can lead to beta-cell apoptosis and diabetes.
- Small molecules that balance ER homeostasis by targeting UPR mediators, ERAD, and aiding protein folding promise efficacy for the treatment of T1D and T2D.

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1 **Title**

2 Pharmacological targeting of endoplasmic reticulum stress in pancreatic beta cells

3 **Authorship**

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14 **Keywords**

15 Diabetes, endoplasmic reticulum stress, beta cell, pharmacology

16 **Abstract**

17 Diabetes is a disease with pandemic dimensions and no pharmacological treatment prevents

18 disease progression. Dedifferentiation has been proposed to be a driver of beta-cell dysfunction

19 in both type 1 and type 2 diabetes. Regenerative therapies aim to reestablish function in

20 dysfunctional or dedifferentiated beta cells and restore the defective insulin secretion.

21 Unsustainable levels of insulin production with increased demand at disease onset strain the

22 beta-cell secretory machinery leading to endoplasmic reticulum (ER) stress. Unresolved chronic

23 ER stress is a major contributor to beta cell loss of function and identity. Restoring ER

24 homeostasis, enhancing ER-associated degradation of misfolded protein, and boosting

25 chaperoning activity, are emerging therapeutic approaches for diabetes treatment.

26

27 **Beta-cell fate in diabetes**

28 Insulin-secreting **beta cells** (see Glossary) and glucagon-secreting **alpha cells** in the pancreatic
29 **islets of Langerhans** cooperate to maintain systemic glucose regulation. In diabetes, blood
30 glucose levels are pathologically elevated due to the destruction or dysfunction of beta cells.
31 Diabetes afflicts well more than 400 million people worldwide. More than 90% of diabetic
32 patients suffer from type 2 diabetes (T2D). T2D is mainly caused by obesity-induced peripheral
33 organ insulin resistance and beta cell's inability to adequately secrete insulin to maintain
34 euglycemia. Type 1 diabetes (T1D; 5–10% of all diabetes cases) is a consequence of
35 autoimmune destruction of beta cells leading to absolute insulin deficiency. Recently, anti-CD3
36 immunotherapy halted T1D for two years [1], but no pharmacological treatment prevents or
37 reverses the loss or dysfunction of beta cells and diabetes progression. Ultimately, T1D and T2D
38 patients require exogenous insulin substitution for survival, but insulin only treats the symptoms
39 (hyperglycemia), but not the root cause (functional beta-cell loss).

40 Instead of generating and transplanting cadaveric human islets [2] or stem cell-derived islets or
41 beta cells [3–5], recent research efforts aim to induce endogenous beta cell regeneration [6].
42 Beta-cell **dedifferentiation** in addition to apoptosis has been proposed as a major cause of
43 functional beta-cell mass loss in diabetes [7,8] (Box 1). Dedifferentiation occurs when
44 environmental and cell-intrinsic factors cause beta cells to lose their identity, glucose-response,
45 and insulin-secretory function [9]. Dedifferentiated beta cells might survive stress-induced
46 insults, but are dysfunctional and cannot adequately regulate blood glucose. Importantly,
47 histological evidence suggests dedifferentiated beta cells exist in both human T1D and T2D [10–
48 13]. To what extent beta-cell dedifferentiation and/or apoptosis contribute to human T1D and
49 T2D progression remains yet to be determined and is highly debated [14,15]. As currently no

50 technology exists monitoring beta-cell mass and function *in vivo*, our knowledge relies on
51 biopsies and cadaveric samples and cannot with certainty pinpoint the underlying
52 pathomechanism of T1D and T2D [16]. In biologically active pancreatic tissue slices, the beta-
53 cell volume of T2D patients was similar to that of body weight-matched non-diabetic individuals
54 [17]. However, T2D tissue slices lacked first and second-phase glucose-stimulated insulin
55 secretion (GSIS). Similarly, pancreatic slices from pre-diabetic glucose-intolerant persons
56 inadequately secreted insulin upon glucose exposure despite conserved beta cell mass [17].
57 Using imaging mass cytometry, Damond et al. showed that human T1D beta-cells lose the
58 expression of key beta-cell makers before immune-cell recruitment and beta-cell destruction
59 [18].
60 Together, these findings suggest that beta-cell dysfunction and dedifferentiation precede T1D
61 and T2D onset and offer a window of opportunity for early intervention. Pharmacological
62 targeting of this remaining beta cell mass to regain its function could prevent disease progression
63 and even reverse diabetes. Therefore, we need to understand the dedifferentiation paths and
64 mechanisms for clinical translation and pharmacological intervention. Here, we review the
65 relevance of **endoplasmic reticulum homeostasis** as a target to reverse the dedifferentiation of
66 remaining dysfunctional beta cells.

67 **Beta-cell ER stress**

68 The ER is the site of secreted and membrane protein synthesis and folding. Approximately one-
69 third of all proteins **translocate** to the ER lumen. This specialized compartment is occupied by a
70 plethora of **chaperones** and enzymes helping proteins to fold properly into their three-
71 dimensional structures. Protein folding is inherently error-prone, leading to **protein misfolding**
72 and aggregation. Accumulation of misfolded proteins can trigger the ER stress response,

73 mediated by the unfolded protein response (UPR) pathway, aiming to increase the capacity of
74 protein synthesis and folding and prevent detrimental stress-related complications.
75 There are three UPR sensors in the ER membrane which have been in detail reviewed elsewhere
76 [19]. Briefly, the three sensors, PKR-like ER **kinase** (PERK), activating **transcription factor** 6
77 (ATF6), and inositol requiring 1 (IRE1) are bound by binding immunoglobulin protein/78-kDa
78 glucose-regulated protein (BiP/GRP78) which is released upon unfolded or misfolded protein
79 accumulation, activating the UPR sensors (Figure 1, Key Figure). However, a BiP-independent
80 mechanism might exist in which misfolded protein directly activates the UPR sensors [20].
81 Activated PERK phosphorylates eIF2α, which in turn inhibits **ternary complex** formation
82 preventing translation initiation. ATF6 is cleaved upon activation and the cytosolic fraction
83 translocates into the nucleus to act as a transcription factor. IRE1α is the ubiquitously expressed
84 isoform of IRE1 and has kinase and endonuclease activity that has high specificity for **splicing**
85 *X-box binding protein 1* (*XBP-1*) mRNA into *XBP-1s* (spliced) isoform.
86 Initially, UPR-activation mitigates ER stress by reducing general protein synthesis as well as
87 inducing factors that enable protein folding (e.g. chaperones) and enhances clearance of
88 misfolded proteins via the ER-associated degradation (ERAD) complex. If this adaptive response
89 is unable to resolve ER stress, cells might become dysfunctional and adaptive UPR can switch to
90 a pro-apoptotic pathway, termed terminal UPR, eventually causing cell death.
91 Beta cells are one of the most efficient protein factories and insulin secretors in the body,
92 producing up to one million proinsulin molecules per minute. The high insulin production rate
93 and the relative complexity of proinsulin to insulin folding poses a huge burden on beta-cell ER
94 homeostasis [21]. In fact, an estimated 10-20% of proinsulin fails maturation and is degraded
95 before secretion [22]. Genetically relieving beta cells from proinsulin biosynthesis mitigates ER

96 stress [23], which indicates that beta cells have a chronically activated UPR to balance ER
97 homeostasis. The genetic deletion of key UPR-mediators such as Ire1 and its target Xbp1 as well
98 as Perk in adult mice causes hyperglycemia and hyperinsulinemia also due to impaired insulin
99 processing in the ER, reviewed in [24]. Also, ER overload with misfolded proinsulin due to
100 genetic mutations in the INS-gene causes unresolvable ER stress that leads to beta cell
101 dysfunction and eventually beta cell death and diabetes in mouse and human [25,26]. Hence,
102 balancing beta-cell ER homeostasis is essential to maintain beta-cell function and blood glucose
103 control. In T1D and T2D, accumulating evidence suggests that disease-induced ER stress
104 contributes to beta-cell failure and diabetes progression [27].
105 The plasma proinsulin-to-C-peptide ratio, a clinical measure for beta-cell ER dysfunction, is
106 pathologically elevated in pre-diabetic as well as T1D patients [28,29]. Beta-cell ER stress
107 marker expression is detected in pre-diabetic non-obese diabetic (NOD) mice and T1D humans
108 [30–32]. T1D pancreatic islet immune cell infiltration appears before frank diabetes and T1D-
109 associated pro-inflammatory cytokines trigger beta-cell ER stress and eventually apoptosis in
110 murine and human pancreatic islets *ex vivo* [33,34]. Recent findings demonstrate the role of beta-
111 cell ER stress for T1D pathogenesis. Beta-cell ER stress of NOD mice converts from protective
112 to deleterious signaling prior progression to overt diabetes [30,31,35]. Deletion of the UPR-
113 sensor Ire1a specifically in beta cells of NOD pups before insulitis develops induced transient
114 beta-cell dedifferentiation. Remarkably, dedifferentiated beta cells were protected against
115 immune-mediated killing and initial hyperglycemia was reversed [36]. However, it remains
116 unknown how these spared beta cells reacquire their function for glucose control in NOD mice.
117 Using a genome-scale CRISPR knockout library to screen for genes that protect beta cells
118 against NOD-mediated immune destruction, Cai et al. identified a protective role for the loss of

119 the human T1D genome-wide association study candidate FAD-dependent amine oxidase
120 Renalase (RNLS) [37]. The deletion of *RNLS* reduced beta-cell ER stress by ameliorating
121 chemical and cytokine-induced UPR activation; making these cells resistant to autoimmune
122 killing [37]. The authors could further show that the FDA approved drug pargyline is a potential
123 RNLS inhibitor, which also protected insulin-producing beta-like cells from human induced
124 pluripotent stem cells (iPSC-beta) against ER stress [37]. Moreover, Leite et al. showed that
125 iPSC-beta cells were only attacked by autologous immune cells from the same donor after
126 chemical ER stress induction *in vitro* [38]. These findings imply a causal role of ER stress in
127 T1D initiation and progression.
128 In T2D patients, histological findings suggest either increased or decreased ER stress marker
129 expression. Thereby, the ER stress level might also depend on disease state and downregulation
130 of UPR-sensors could indicate an impaired response to the increased insulin biosynthesis
131 demand due to worsening insulin resistance [39–41]. In addition, T2D-associated chronically
132 elevated glucose and lipid levels might further disturb the ER folding milieu by UPR-activation
133 potentially contributing to impaired proinsulin folding and beta-cell dysfunction [42]. ER stress
134 marker gene expression of isolated islets is specifically upregulated at disease onset in leptin-
135 deficient ob/ob and leptin receptor-deficient db/db mice, which are both used to model
136 hyperphagia-induced beta-cell failure in T2D [43]. Recently, Arunagiri et al. showed that
137 proinsulin misfolding is an early event in T2D progression of db/db mice [44]. Moreover, they
138 showed that proinsulin aggregates emerge also in human islets with a chemically perturbed ER
139 folding environment [44].
140 Collectively, accumulating evidence demonstrates beta-cell ER stress contribution to beta-cell
141 dysfunction in T1D and T2D early in disease pathology even before overt diabetes.

142 Pharmacological targeting of ER stress to restore ER homeostasis is a promising approach to
143 prevent or reverse diabetes progression. Here, we review emerging pharmacological approaches
144 to restore ER homeostasis of dysfunctional beta cells.

145 **Pharmacological targeting of beta-cell stress pathways**

146 *Modulating the PERK branch*

147 PERK is one of the four kinases that phosphorylate the eukaryotic initiation factor 2α (eIF2α)
148 causing the inhibition of translation initiation to reduce the ER load (Figure 1). Conversely,
149 phosphorylation of eIF2α favors the translation of some stress-induced mRNAs, including the
150 transcription factor *ATF4*, which in turn upregulates CHOP. CHOP deletion in db/db mice
151 enhances the adaptive UPR and oxidative stress pathways and downregulates pro-apoptotic
152 genes, leading to a compensatory increase in beta-cell mass [45]. The effect of PERK inhibitors
153 on beta cells was investigated as a potential target for increasing ER capacity and reducing
154 deleterious effects of UPR activation. Surprisingly, pharmacological inhibition of PERK by
155 GSK2606414 [46], tested for neuroprotective effects in mice, leads to hyperglycemia [47].
156 However, when the PERK inhibitors GSK2606414 and GSK2656157 were administered at a low
157 dose, allowing for partial eIF2α phosphorylation, GSIS was enhanced in hyperglycemic mice
158 [48,49]. The downstream effectors *Atf4* and *Chop* were not affected but the chaperone *Bip* was
159 induced by low-dose GSK2606414 treatment [49]. These results suggest that excessive PERK
160 inhibition prevents an appropriate physiological UPR response but low-level inhibition has a
161 positive effect on insulin secretion. Therefore, as a pharmacological tool, PERK inhibitors have
162 to be carefully dosed. Interestingly, also excessive eIF2α dephosphorylation inhibitors worsen
163 beta-cell function. Preventing general translation by constant phosphorylation of eIF2α prevents
164 insulin translation and activates the downstream UPR. Salubrinal, an inhibitor eIF2α

165 dephosphorylation, maintains high p-eIF2α levels, which become toxic to beta cells [50].

166 Guanabenz, another eIF2α dephosphorylation inhibitor, does not cause a direct toxic effect in

167 beta cells, as p-eIF2α levels do not reach critical levels, but at the same time, it prevents

168 protective measures against fatty-acid induced ER stress [51]. These results imply that

169 pharmacological modulation of PERK and eIF2α must allow for preserving physiological

170 signaling through the PERK branch.

171 *Modulating the ATF6 branch*

172 ATF6 is the least studied UPR sensor and likely the most versatile one. ATF6 and its molecular

173 roles have been reviewed in detail [52]. The cleaved ATF6-N binds the *cis*-acting ER-stress

174 response element in mammals, which directly induces chaperones, *XBP-1* mRNA, and *CHOP*

175 mRNA [53] (Figure 1). The PERK and ATF6 branches cooperatively induce CHOP and shift the

176 cell fate towards apoptosis. However, ATF6 is also important in a more adaptive response

177 through chaperone and antioxidant induction and has been shown to induce ER-stress

178 independent cellular proliferation, likely through mTOR. Aft6α-deletion improves insulin

179 sensitivity in insulin-resistant and obese mice, whereas it reduces pancreatic insulin content in

180 Akita mice [54]. Another study found that ATF6 drives beta-cell proliferation under mild

181 glucose-induced ER stress in vitro in murine and human islets [55]. ATF6-activating compounds

182 have been identified through high-throughput screening approaches [56,57]. Compound 147,

183 identified in this screen, protects against cardiac, kidney, and neurological damage in mice by

184 restoring ER homeostasis [58]. The effects of ATF6-activating compounds have not been

185 reported in beta cells to date. At the same time, ATF6 inhibitors, so-called Ceapins were

186 identified [59], as potential anti-cancer drugs that have however not been tested *in vivo* yet.

187 Nelfinavir, an anti-HIV drug that inhibits ATF6 proteolytic activation, decreases GSIS in INS-1E
188 rat beta-cell line [60], showcasing the positive adaptive role of ATF6 in beta cells.
189 *Modulating the IRE1 branch*
190 IRE1α is an ER transmembrane protein with a substrate-binding luminal domain and two
191 cytosolic enzymatic activities: a kinase and an **endoribonuclease** [61]. Under ER stress, IRE1α
192 oligomerizes causing juxtaposing and trans-autophosphorylation of the cytosolic kinase domain
193 (Figure 1). Pioneering work led by Feroz Papa and colleagues delivered mechanistic evidence for
194 a key role of IRE1α in mediating a life and death decision of beta cells [62].
195 Remediable ER stress causes low-level IRE1α oligomerization that restricts IRE1α's
196 endoribonuclease activity to *XBP1* mRNA splicing. Spliced *XBP1* encodes the XBP1s
197 transcription factor whose target genes enhance ER protein folding capacity, supporting ER
198 homeostasis and cell survival. Unresolved ER stress, however, causes sustained, high-level
199 kinase autophosphorylation and subsequently higher-order IRE1α-oligomerization. This
200 conformational change hyperactivates IRE1α's ribonuclease (RNase) activity and causes massive
201 endonucleolytic degradation of ER-localized mRNAs, termed Ire1-dependent decay (RIDD)
202 [63,64]. In beta cells, RIDD activity causes loss of beta-cell identity by the degradation of e.g.
203 insulin mRNAs as well as essential ER-resident molecular chaperones, oxidoreductases, and
204 glycosylating enzymes [63]. Eventually, ER stress-induced hyperactive IRE1α causes beta-cell
205 apoptosis, which is independent of *XBP1* mRNA splicing [31,63,65,66].
206 Small molecules termed KIRA's (kinase-inhibiting RNase-attenuators) break IRE1α oligomer
207 formation and attenuate pro-apoptotic RIDD while largely maintaining ER-homeostatic XBP1
208 splicing [31,66–68]. KIRA6 ameliorates beta-cell ER stress-induced IRE1α hyperactivation in
209 the Akita mouse, increasing beta-cell survival and function to improve glucose and insulin

210 metabolism [66]. KIRA8 (formally called compound 18 developed by Amgen) is a highly potent
211 and selective IRE1α kinase inhibitor with a preferable pharmacokinetic profile that enables daily
212 **intraperitoneal** dosing, thereby requiring less frequent dosing than KIRA6 (once vs. twice
213 daily) [31,69]. Similar to KIRA6, KIRA8 ameliorates beta-cell ER-stress induced diabetes of
214 Akita mice [31]. In NOD mice, KIRA8 not only preserved beta-cell mass and function by
215 ameliorating ER stress-induced beta-cell RIDD but also reversed diabetes progression [31].
216 KIRAs are still under preclinical testing and undergo chemical improvements to enhance
217 **bioavailability** and solubility [62]. Collectively, these promising results demonstrate that
218 directly targeting IRE1α to attenuate its pro-apoptotic RIDD while permitting XBP1 splicing
219 beneficially affects beta-cell survival and function in preclinical diabetic models.
220 The ABL-family kinases modulate the IRE1α oligomerization state [31,70]. Imatinib, also
221 known as Gleevec, an anti-cancer tyrosine kinase inhibitor, prevents ER stress-mediated
222 activation of c-Abl, which avoids co-localization of c-Abl with IRE1α at the ER membrane and
223 thus high-order IRE1α oligomerization [31]. This could be one mechanism of imatinib's anti-
224 diabetes efficacy in preclinical models [31,63,71,72] and supports current Phase II clinical
225 testing of imatinib for the treatment of new-onset T1D (NCT01781975). These results
226 demonstrate the potential of modulating the IRE1 activation state to ameliorate beta cell ER
227 stress for diabetes therapy.
228 *Modulating ER-associated degradation*
229 ERAD is the only known mechanism in the cell dedicated exclusively to the disposal of
230 misfolded proteins in the ER (Figure 1). The biochemical processes of ERAD were subject to
231 intensive research in the last two decades and have been reviewed in detail elsewhere [73,74]. In
232 principle, ERAD consists of three distinct yet coordinated steps: (1) substrate recognition and

233 targeting to the retro-translocon, (2) substrate **retro-translocation** from the ER lumen to the
234 cytosol, and (3) ubiquitin – **proteasome**-dependent substrate degradation [73].
235 The Sel1l-Hrd1 ERAD-complex is the most conserved ERAD complex in mammals. Hrd1 is a
236 multi-spanning ubiquitin ligase (E3) with a cytosolic RING finger domain. The recently resolved
237 cryo-electron microscopy structure of S. cerevisiae Hrd1 suggested that five of Hrd1's eight
238 transmembrane helices form an aqueous cavity extending from the cytosol almost to the ER
239 lumen [75]. According to this structure, Hrd1 might form a retro-translocation channel for
240 misfolded polypeptides through the ER membrane [75,76].
241 Sel1l is indispensable for Hrd1 stabilization, mammalian ERAD, and thus ER homeostasis
242 [77,78]. The importance of mammalian ERAD is demonstrated by the embryonic lethality of
243 mice with germline deletion of Sel1l or Hrd1 [79,80] and premature death after acute deletion of
244 either gene [77,81]. Beta-cell specific deletion of Sel1l using RIP-Cre and Ins1-Cre mice
245 revealed that Sel1l-Hrd1-ERAD function is indispensable for beta cell identity and function *in*
246 *vivo* [82,83]. In both models, hyperglycemia and glucose intolerance is caused by beta-cell
247 dysfunction and not a decline in beta cell mass [82,83]. RNA-transcriptomics (bulk RNA
248 sequencing as well as single-cell RNA sequencing) of pancreatic islets of Ins1-Cre Sel1l-
249 deficient mice revealed beta-cell dedifferentiation with increased immaturity marker expression
250 and reduced expression of beta-cell maturity markers [83]. Furthermore, scRNA-seq identified
251 beta-cell TGFβ receptor 1 (TGFβR1) as ERAD-target with increased TGFβR1 expression in
252 isolated Sel1l-deficient islets [83]. TGFβR1-specific antagonism increased beta-cell maturity
253 marker expression and Sel1l-islet insulin content implying beta-cell redifferentiation *ex vivo*
254 [83]. These results not only reinforce targeting TGFβ signaling for diabetes treatment [84,85] but
255 also position ERAD as a drug target for improving beta-cell health and function.

256 The female sex hormone estrogen, via nuclear estrogen receptor alpha activation, stimulates
257 ERAD-mediated ER stress relief of beta cells in Akita mice leading to beta-cell protection and
258 diabetes amelioration [86]. Estrogen's beneficial effects on beta-cell protection have also been
259 shown in clinical trials [87]. However, deleterious gynecological, oncogenic, and mitogenic side
260 effects preclude the use of chronic estrogen therapy in diabetes. We recently demonstrated that
261 stable Glucagon-like peptide 1/estrogen conjugation (GLP-1/estrogen) enables delivery of the
262 steroid cargo specifically to GLP-1 receptor (GLP-1R) expressing beta cells of pancreatic islets
263 [88]. The selective delivery of estrogen to beta cells decreased daily insulin requirements by
264 60%, triggered estrogen-specific activation of ERAD, and further increased beta-cell survival,
265 proliferation, redifferentiation leading to beta cell regeneration in multiple low dose
266 streptozotocin (mSTZ)-diabetic mice [88]. GLP-1/estrogen circumvents estrogen's detrimental
267 effects in GLP-1R negative tissues (i.e. breast and uterus) of mice and rats and did not show any
268 signs of systemic toxicity [88,89], a prerequisite for any future clinical testing.
269 Altogether, these findings support the importance of ERAD in beta cell viability and function.
270 Estrogen is the only known substance to increase ERAD activity *in vivo*. Developing
271 pharmacological tools that enhance misfolded protein degradation without directly interfering
272 with UPR function will most likely play an important role in managing ER-associated diseases.
273 *Chaperones*
274 Efforts to pharmacologically modulate the UPR sensor branches aim for shifting the balance
275 from pathogenic to physiological or adaptive UPR activity. Another pharmacological approach is
276 enhancing the chaperone response and folding activity in beta cells, circumventing deleterious
277 UPR activation, and preventing unwanted shifts in homeostasis. To enhance chaperone action,

278 two promising pharmacological approaches emerged – pharmacological chaperones (PCs)
279 [90,91], and chemical chaperones.
280 PCs are small molecules that bind to the unfolded form of a specific target. They are used to aid
281 mutant protein folding in genetic diseases, such as cystic fibrosis and lysosomal storage
282 disorders, or a specific misfolded protein. Moreover, PCs can counteract the dominant-negative
283 effects and damage caused by misfolded wild-type proteins. However, off-target effects have to
284 be carefully considered [92].
285 Sulfonylureas are used in diabetes treatment as inhibitors of sulfonylurea receptor 1 (SUR1), a
286 subunit of the pancreatic ATP-sensitive potassium channel. Sulfonylureas also rescue SUR1
287 folding defects and aid trafficking of mutant SUR1 to the cell surface in patients with congenital
288 hyperinsulinism in vitro, thus acting as PCs [93,94]. Another PC, termed O4, prevents *in vitro*
289 aggregation of the hormone islet amyloid polypeptide (IAPP), a hallmark of T2D [95].
290 Chemical chaperones, on the other hand, are compounds that aid protein folding in a non-specific
291 manner. **Amphiphilic** chemical chaperones bind the exposed hydrophobic parts of unfolded and
292 misfolded proteins, thereby preventing protein aggregation and ER stress. Currently, clinical
293 trials for diabetes treatment with the chemical chaperone Tauroursodeoxycholic acid (TUDCA)
294 is ongoing (NCT02218619). TUDCA is a Food and Drug Administration (FDA)-approved drug
295 for the treatment of primary biliary cirrhosis. Another chemical chaperone, sodium
296 phenylbutyrate (PBA) is FDA-approved for the treatment of urea cycle disorders. Both
297 compounds have on top of the chaperone activity, secondary positive effects on cellular
298 homeostasis. PBA transiently induces heat-shock protein expression in a bronchial epithelial cell
299 line [96] and TUDCA activates AKT signaling in mesenchymal stem cells [97]. PBA and
300 TUDCA are both promising drug candidates to restore cellular homeostasis in beta cells.

301 **Concluding Remarks and Future Perspectives**

302 Both T1D and T2D are characterized by beta-cell dysfunction early in disease progression. Beta
303 cells are well adapted to their secretory function, albeit they remain highly sensitive to cellular
304 stress. The accumulation of unfolded and misfolded protein in the ER leads to UPR activation
305 and ER stress. The activity of the UPR and related compensatory mechanisms are inevitable for
306 proper beta-cell function.

307 ER stress-related pathways are promising pharmacological targets to restore beta-cell function.
308 Our understanding of the role of the three UPR branches PERK, ATF6, and IRE1α from mouse
309 models allows for molecular target selection and drug screening. PERK signaling activators and
310 inhibitors perturb the delicate mechanism of misfolded protein sensing and translation inhibition
311 and result in defective unfolded protein adaptation. The pharmacological alteration of the
312 downstream PERK signal requires very precise dosing and further preclinical investigation.
313 ATF6 is mainly associated with chaperone induction, adaptation, and survival in beta cells.
314 ATF6 activating compounds were developed with the aim of acting as pro-survival substances in
315 a variety of diseases accompanied by protein misfolding [98]. IRE1α is responsible for
316 chaperone expression and a variety of downstream adaptive responses. Under chronic stress
317 conditions, IRE1α drives apoptosis through RIDD. The KIRA inhibitors prevent pro-apoptotic
318 IRE1α oligomer formation while maintaining IRE1α's function in adaptive UPR. For the
319 pharmacological modulation of the UPR sensors, human translation is required to overcome the
320 remaining obstacles in bioavailability, solubility, and safety. These issues might be partially
321 overcome by targeted delivery by using beta-cell specific markers, such as recently demonstrated
322 by us, using GLP-1 peptide-mediated beta-cell targeting [88]. Future preclinical studies as well
323 as human transplantation studies are needed to pursue first clinical trials in diabetic patients. In

324 summary, drugs altering the UPR response have the potential to improve beta-cell function,
325 under the requirement that they do not alter the cell's ability to respond to cellular stress.
326 Combinatorial drug therapies targeting different branches of UPR and ERAD might improve
327 beta-cell function by fine-tuning ER homeostasis (see Outstanding Questions).
328 Pharmaceutical and chemical chaperones enhance the cell's compensatory mechanisms and can
329 prevent a UPR response, aiding folding and restoration of homeostasis. The chemical chaperone
330 TUDCA is currently in a clinical trial, as it has proven safe in the treatment of other diseases.
331 Pharmaceutical chaperones targeting IAPP and misfolded proinsulin, could hypothetically
332 selectively shield from deleterious effects of misfolded proinsulin accumulation in beta cells.
333 In summary, we believe that drugs aimed at increasing beta-cell protection and/or function
334 through lowering ER stress and enhancing compensatory mechanisms through chaperones will
335 prove to be an efficient therapy in combination with other approaches for the treatment of
336 diabetic patients.

337 **Glossary**

338 Alpha cell: an endocrine cell type in the islets of Langerhans that produces the hormone
339 glucagon, which regulates glucose release into the bloodstream
340 Amphiphilic: containing a hydrophobic and a hydrophilic part
341 Beta cell: an endocrine cell type in the islets of Langerhans that produces the hormone insulin,
342 which regulates glucose uptake from the bloodstream
343 Bioavailability: the fraction of a drug that enters the bloodstream
344 Chaperone: molecular chaperones are proteins that facilitate protein folding and unfolding,
345 chemical and pharmacological chaperones are synthetic molecules with the same function
346 Dedifferentiation: the process of acquiring a less mature cellular phenotype

347 Endocrine progenitor: a precursor of a cell producing and secreting hormones into the

348 bloodstream

349 Endoplasmic reticulum: a cellular organelle important in secreted and membrane protein

350 synthesis, folding, modification, and trafficking, calcium homeostasis, lipid synthesis

351 Endoribonuclease: an enzyme that catalyzes an internal cleavage of RNA

352 Homeostasis: a state of cellular or physiological equilibrium maintained by an organism

353 independent of external conditions

354 Intraperitoneal: administered into or within the abdominal cavity

355 Islets of Langerhans: groups of tightly packed endocrine cells located in the pancreatic tissue

356 Kinase: an enzyme that facilitates phosphorylation, i.e. the transfer of a phosphate group

357 Maturation (cellular): the cellular process of acquiring a more functional phenotype and typically

358 losing proliferative potential

359 Proteasome: a protein complex that digests proteins labeled with ubiquitin into short peptides

360 Protein misfolding: failure to acquire a correct three-dimensional conformation, the exposed

361 hydrophobic domains are prone to aggregation

362 Retro-translocation: peptide chains are exported from the ER to the cytosol for degradation

363 Splicing: a molecular process in RNA maturation, when non-coding introns are cleaved out from

364 the protein-coding sequence of RNA

365 Ternary complex (translation initiation): eIF2 binds GTP and the methionine initiator tRNA to

366 form a ternary complex, and accompanied by other factors allows the ribosome to scan the

367 mRNA for the start codon

368 Transcription factor: a protein that binds DNA and alters the expression of a gene

369 Translocation into the ER: the newly synthesized peptide chain is inserted into the ER membrane
370 or ER lumen through a channel

371 **Text Boxes**

372 *Box1: Beta-cell dedifferentiation*

373 Beta-cell differentiation and **maturation** are regulated by timed expression levels of a complex
374 transcription factor (TF) network, such as *PDX1*, *NEUROG3*, *NKX6.1*, and *FOXO1* [99]. Beta
375 cells sense glucose levels and as a response adequately secret insulin to keep blood glucose
376 within a narrow range (~4-6mM in fasting adult humans). In experimentally induced rodent
377 models of hyperglycemia, pancreatic islets lose the expression of beta cell-specific key TFs and
378 maturity markers [7,100,101]. In 2012, Accili and colleagues proved the beta-cell specific loss of
379 identity marker expression by genetic lineage tracing *in vivo* [8]. Deleting beta cell-specific
380 *Foxo1* expression, they showed that instead of accelerated beta-cell apoptosis, beta cells of these
381 mice acquired a dysfunctional more embryonic-like phenotype, which caused insulin-deficient
382 diabetes. Beta-cell maturity makers and TFs (e.g. *Insulin*, *Slc2a2*, *Nkx6.1*, and *Pdx1*) were down-
383 regulated while TFs marking pluripotency or **endocrine progenitors** were up-regulated
384 (*Neurog3*, *Oct4*, *Nanog*). This altered beta-cell identity was initially termed beta-cell
385 dedifferentiation and refers to the reversal of the beta-cell differentiation state [8,102,103].
386 However, there has been ambiguity in defining beta-cell dedifferentiation, often used to describe
387 general beta-cell phenotype alterations in diabetes. There is a debate to whether β-cell
388 dedifferentiation resembles a reversal to an endocrine progenitor state, which might depend on
389 the experimental model and/or hyperglycemia severity: high blood glucose (> 33 mM) induce
390 Ngn3 expression in murine models [8,104], whereas lower blood glucose does not
391 [7,13,88,100,105]. The altered beta-cell identity has also been described as beta-cell

392 degranulation, where diabetic stress depletes insulin granules [12,105,106]. However, it remains
393 unclear whether degranulated beta cells are in fact “empty” or just appear so due to undetectable
394 insulin protein expression by standard immunohistochemistry [10,12]. Glycemia normalization
395 reversed β-cell degranulation accompanied by increased insulin expression in a genetic mouse
396 model of diabetes [105]. Hence, dedifferentiated and degranulated beta cells potentially refer to
397 the same dysfunctional beta-cell state and rather refer to the method (ultrastructural or mRNA
398 expression profiling) with which they are determined. The stress-induced phenotypic alterations
399 of beta cells can also promote the expression of other endocrine hormones (such as glucagon) by
400 loss of lineage determining or gain of lineage inappropriate factors in both diabetic animals as
401 well as human T1D and T2D [10–12,88]. Regardless of its definition, these findings highlight
402 beta-cell plasticity especially in a diseased state with emerging evidence for altered beta-cell
403 identity in human T1D and T2D. Importantly, the redifferentiation of dedifferentiated beta cells
404 could constitute a new therapeutic approach for diabetes.

405 **Figure Legends**

406 *Figure 1: Pharmacological regulation of the UPR and ERAD pathways.*

407 Secreted and membrane proteins are cotranslationally translocated into the ER lumen or inserted
408 into the membrane, respectively. Folding of the newly synthesized proteins is aided by molecular
409 chaperones. When misfolded protein accumulates, the three UPR sensors IRE1α, PERK, and
410 ATF6 are activated and ERAD is enhanced. Chemical and pharmaceutical chaperones
411 complement the function of molecular chaperones and prevent aggregation and facilitate folding.
412 Specific inhibitors or activators of the UPR sensors direct the cell fate towards an adaptive UPR
413 response and survival. ERAD enhancers accelerate misfolded protein degradation, prevent
414 aggregation, and resolve ER overload.

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640

Outstanding Questions Box

- Is ER stress the cause or consequence of beta-cell dysfunction?
- Is it possible to design small molecules that specifically inhibit apoptotic signals while maintaining physiological UPR function?
- What effects does combining multiple compounds that target different arms of the UPR response and/or ERAD have?
- To which misfolding-prone targets can pharmaceutical chaperones be developed?
- Despite being beneficial in beta cells, what are the potential side effects of systemic pharmacological ER stress modulation?

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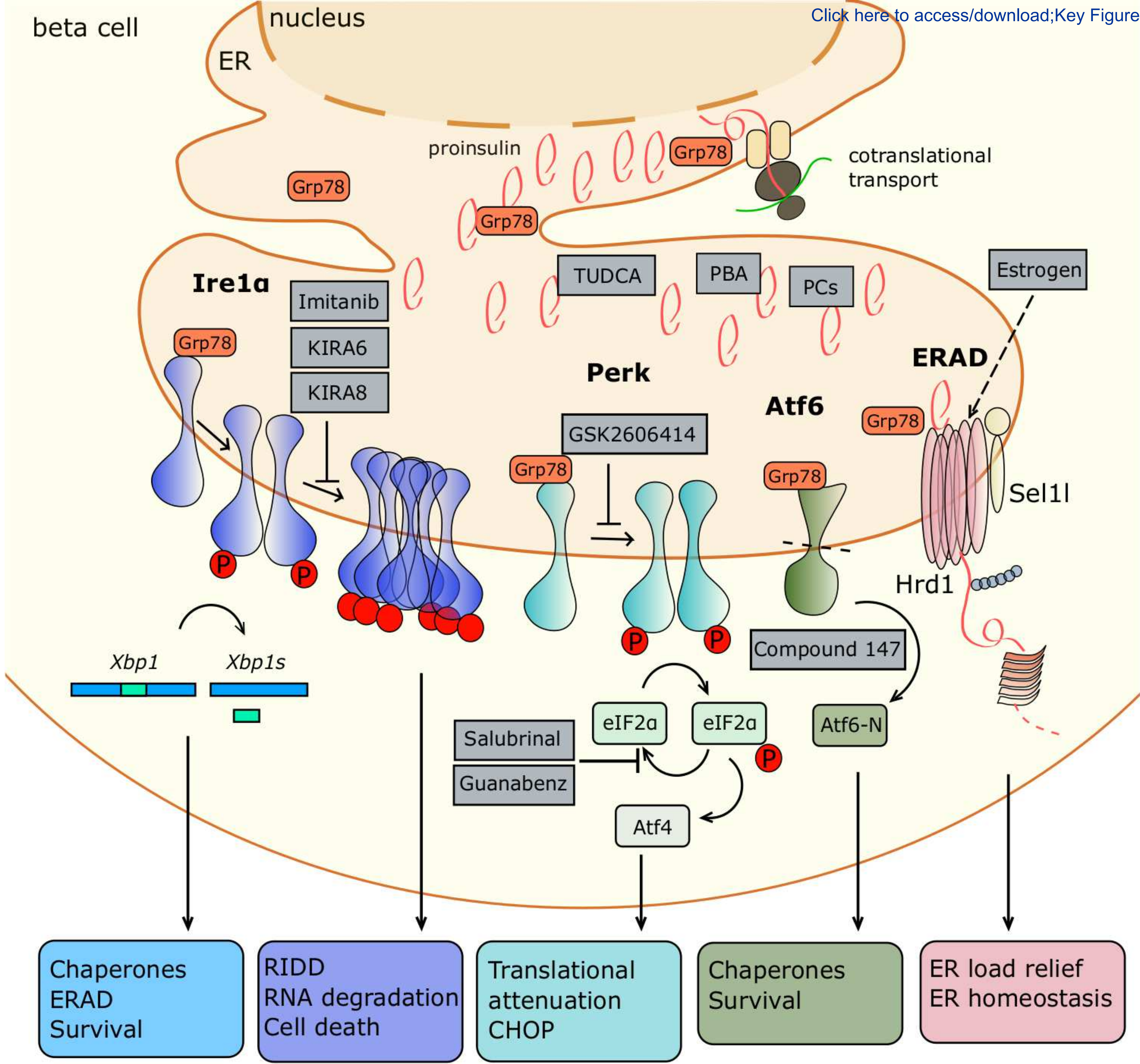

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