King's College London

King's Research Portal

DOI:
10.1055/a-2190-2803

Document Version
Publisher's PDF, also known as Version of record

Link to publication record in King's Research Portal

Citation for published version (APA):
Schepp, F., Schubert, U., Schmid, J., Lehmann, S., Latunde-Dada, G. O., Kose, T., Steenblock, C., Bornstein, S. R., Linkermann, A., & Ludwig, B. (2023). Mechanistic Insights into Ferroptotic Cell Death in Pancreatic Islets. *Hormone and Metabolic Research*. Advance online publication. https://doi.org/10.1055/a-2190-2803

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
•You may not further distribute the material or use it for any profit-making activity or commercial gain
•You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 26. Apr. 2025

Mechanistic Insights into Ferroptotic Cell Death in Pancreatic Islets

Florian Schepp [1], Undine Schubert [2 3 4], Janine Schmid [2 3 4], Susann Lehmann [2 3 4], Gladys Oluyemisi Latunde-Dada [5], Tugba Kose [5], Charlotte Steenblock [2 4], Stefan R Bornstein [2 3 4 5 6 7], Andreas Linkermann [8 9], Barbara Ludwig [2 3 4 6]

Florian Schepp 1 Klinik für Viszeral-, Thorax- und Gefäßchirurgie, Universitätsklinikum Carl Gustav Carus an der TU Dresden, Dresden, Germany

Undine Schubert 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany 3 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus and Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

Janine Schmid 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany 3 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus and Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

Susann Lehmann 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany 3 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus and Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

Gladys Oluyemisi Latunde-Dada 5 Division of Diabetes & Nutritional Sciences, School of Life Course and Population Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom of Great Britain and Northern Ireland

Tugba Kose 5 Division of Diabetes & Nutritional Sciences, School of Life Course and Population Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom of Great Britain and Northern Ireland

Charlotte Steenblock 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

Stefan R. Bornstein 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany 3 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus and Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.), Neuherberg, Germany 5 Division of Diabetes & Nutritional Sciences, School of Life Course and Population Sciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom of Great Britain and Northern Ireland 6 CRTD, DFG-Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany 7 Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

33 Andreas Linkermann 8 Division of Nephrology, Department of Medicine III, University Hospital Carl Gustav Carus, Technische
34 Universität Dresden, Dresden, Germany 9 Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine,
35 Bronx, New York, United States

36 Barbara Ludwig 2 Department of Medicine III, University Clinic Carl Gustav Carus, Technische Universität Dresden, Dresden,
37 Germany 3 Paul Langerhans Institute Dresden of the Helmholtz Center Munich at the University Hospital Carl Gustav Carus
38 and Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany 4 Center for Diabetes Research (DZD e.V.),
39 Neuherberg, Germany 6 CRTD, DFG-Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden,
40 Germany

41

42

43 Introduction

44 Ferroptosis was identified as a non-apoptotic cell death mechanism, which causes iron-
45 dependent peroxidation of membrane lipids and subsequent membrane rupture [1,2] As a
46 form of necroptotic cell death, ferroptosis has already been shown to play an important role in
47 the pathogenesis of various diseases [3]. Ferroptosis can be experimentally induced by the
48 glutathione peroxidase-4 (GPX4) inhibitor RSL3. The inhibition of GPX4 causes oxidative
49 stress through the release of free iron molecules and induction of lipid peroxidation, followed
50 by a membrane burst with massive necrotic inflammation [1,4]. The effects of ferroptotic cell
51 death can be attenuated by the treatment with ferrostatin-1 (Fer-1) [5,6], a small lipophilic
52 molecule with various functions. Fer-1 mainly acts as an iron scavenger and prevents lipid
53 peroxidation and subsequent membrane rupture [5]
54 The transplantation of pancreatic islets into the liver represents a therapeutic strategy mainly
55 for patients with type 1 diabetes (T1D) experiencing life-threatening hypoglycemic situations
56 despite optimal diabetes therapy or, in the case of an autologous transplantation setting, to
57 prevent patients undergoing (sub-)total pancreatectomy from iatrogenic diabetes [5,7,8]. The
58 intraportal transplantation of pancreatic islets is less invasive than a complete pancreas
59 transplantation but has comparable effects on the stabilization of glycemic control and
60 diabetes-associated complications [8,9]. However, it is estimated that nearly 70% of the islet
61 graft is lost during the peri- and post-transplant period [10].

62 To further improve the outcome of this therapy, it is of key importance to minimize islet loss
63 and consequently improve islet functional potency and survival. A better understanding of
64 mechanisms mediating islet death during and after the isolation process and targeted
65 prevention are therefore key issues. Bruni *et al*. first described in 2018 that human pancreatic
66 islets are susceptible to pharmaceutically induced ferroptosis. The aim of our study was to
67 gain closer insight into the mechanism of ferroptosis in pancreatic islets on a cellular and
68 functional level and thereby identify potential interventional strategies.
69

Materials and Methods

Islet isolation

Pancreatic islets were isolated from female Wistar rats according to guidelines established by the University of Dresden Institutional Animal Care and Use Committee. Animals were euthanized with CO_2. Afterwards, the abdomen was opened, and the pancreatic duct was clamped at the papilla. The digestion solution (1 mg/ml collagenase V (Sigma-Aldrich) and 100 µg/ml DNase (Roche)) was injected *in situ* into the pancreas via the bile duct. The pancreas was carefully dissected and transferred to digestion solution. The digestion was supported by gentle shaking at 37°C for approximately 12 min. After adding cold washing solution (RPMI 5.5 mM glucose, 10% FBS), the digest was filtered through a cell strainer with a pore size of 600 µm (Sigma-Aldrich) and centrifuged for 1 min (277g, Acc6, Dec6, 4°C). The washing procedure was repeated three times. Pancreatic islets were then separated from exocrine tissue by discontinuous density gradient centrifugation (15 min, 1590g, Acc2, Dec2, 4°C) using Ficoll (Sigma-Aldrich) density layers of 1.125 g/cm³, 1.096 g/cm³, 1.08 g/cm³ and 1.06 g/cm³. The interfaces containing the purified islets were collected, washed and cultured in 5.5 mM glucose RPMI 1640 (PAA) supplemented with 10% FBS, 20 mM HEPES, 1x penicillin-streptomycin at 37°C in a 5% CO_2 atmosphere prior to further experimentation.

Islet yield and purity

Dithizone (DTZ), a zinc chelating agent, is known to selectively stain the islets of Langerhans in the pancreas. While exocrine tissue is not stained, the endocrine part appears red. For determination of the islet yield islet particle number (IP) and islet equivalents (IEQ, islet volume normalized to 150 µm diameter) were determined. To this end, representative triplicate samples were stained with dithizone (2% w/v DTZ in 0.25% v/v DMSO in phosphate-buffered saline (PBS)) and examined using an inverted microscope equipped with a 10x objective and eyepiece micrometer. All islets with a diameter >50 µm were grouped into diameter classes of 50 µm segments (i.e., 50–100, 100–150, 150–200, etc.). Each diameter class was converted into the mean volume of 150-µm diameter islets by a relative conversion factor. This method enables the evaluation of the total IEQ number and total IP number of each preparation. The purity and morphology were described descriptively using light microscopy observation at 100X magnification.

Islet viability

Islet viability was assessed by double staining with fluorescein diacetate (FDA) and propidium iodide (PI). FDA is a non-fluorescent molecule, which is hydrolysed to green-fluorescent fluorescein in live cells. Dead cells cannot accumulate or hydrolyse FDA. PI only permeates

106 through the membrane of dead cells resulting in red fluorescence. In detail, islets were washed
107 with PBS and both agents, FDA and PI, were added at a final concentration of 0.5 and 75 µM,
108 respectively. Samples were incubated in the dark for 5 min and evaluated using an inverted
109 fluorescence microscope (100x magnification). For each sample, 100 islets were individually
110 analyzed by calculating the percentage of non-viable cells (red) and viable cells (green). Islets
111 were grouped into 5 categories (0%, 25%, 50%, 75%, and 100% viability) and islet viability
112 was calculated.
113

114 **Glucose-stimulated insulin secretion**

115 The functional secretory capacity of pancreatic islets was analyzed by glucose-stimulated
116 insulin secretion. Therefore, islets were transferred to Modified Krebs Ringer Buffer (MKRB)
117 and equilibrated at 3.3 mM glucose at 37°C for 30 min. Afterwards, islets were divided into
118 two groups containing fresh MKRB with either 3.3 mM or 16.7 mM glucose and incubated for
119 1 h in a gently shaking water bath at 37°C. For both conditions, five samples containing ten
120 islets each were used. After incubation, islets were collected by gentle centrifugation at 200 g
121 for 1 min. Secreted insulin in the supernatants was measured by ELISA (Mercodia Insulin
122 Elisa Kit). Pellets were resuspended in 200 µl PBS and analyzed for DNA content determined
123 by DNeasy Kit (Qiagen). Stimulation Index (SI) is calculated as the ratio of secreted insulin in
124 high glucose in comparison to low glucose, both normalized to DNA content (SI = [$Insulin_{stim}$/
125 total DNA_{stim}] / [$Insulin_{res}$ /total DNA_{res}].
126

127 **Lipid peroxidation**

128 Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage,
129 typically by reactive oxygen species, and is a useful marker for oxidative stress. The
130 peroxidation process leads to the production of malondialdehyde (MDA), which can be
131 measured using the Malondialdehyde Microplate Assay Kit (Cohesion Biosiences). The lipid
132 peroxidation was determined by the reaction of MDA with thiobarbituric acid (TBA) to generate
133 the MDA-TBA adduct. The MDA-TBA adduct was quantified colorimetrically (λ = 532 nm and
134 600 nm). MDA levels were calculated according to the manufacturer's instruction.
135

136 **Zinc and iron measurement**

137 For the Inductively coupled plasma mass spectrometry analysis (ICP-MS), the islets were
138 thawed and centrifuged. The supernatant was discarded, and the pellet resuspended in 200
139 µl 50 µM NaOH. For the physical cell lysis, the islets were drawn up six times with a syringe
140 and spread out again. Then 50 µl of each sample was used for protein quantification. The
141 remaining 150 µl was placed in a concentrator at 60°C for 4 h. Afterwards, 200 µl HNO_3 was

142 added to resuspend the cell extract by thorough vortexing. To complete the digestion, the
143 samples were heated for further 4 h at 80°C.
144 After digestion, the samples were cooled to room temperature overnight. 2.6 ml of HPLC water
145 was added to the samples and the iron concentration was analyzed by ICP-MS.
146

147 **Islet treatment with Fer-1 over a period of 7 days**

148 After isolation, all islets were aliquoted equally into two treatment groups: (i) culture media
149 with vehicle (DMSO) as solvent control and (ii) culture media with 10 µM Fer-1 (Ferrostatin-1,
150 Merck Millipore, 341494). Readout assays were performed on days 3, 5, and 7.
151

152 **RSL3 dilution series**

153 After isolation, all islets were aliquoted equally into five groups with increasing concentrations
154 of RSL3 (5 µM, 10 µM, 15 µM, 20 µM, 40 µM; RSL3, type 2 FIN, Selleck Chemicals, S8155)
155 and a solvent control with DMSO. After 24 h FDA/PI assay was performed, and viability was
156 determined.
157

158 **Ferroptosis inhibition**

159 Islets were divided equally into four treatment groups: (i) culture media with vehicle (DMSO)
160 as solvent control, (ii) culture media with a single treatment of 10 µM Fer-1 and day 0, (iii)
161 culture media with a single treatment of 20 µM RSL3 on day 1, (iiii) culture media with a
162 combination of 10 µM Fer-1 on day 0 and 20 µM RSL3 on day 1. The day of isolation was
163 defined as day 0. All readouts were performed on day 2 after isolation.
164

165 **Immunohistochemical analysis**

166 For immunohistochemical analysis, 80-100 islets were fixed in 4% paraformaldehyde (PFA)
167 for at least 1 hr, washed with PBS and embedded in tissue-Tek O.C.T. (Sakura Finetek).
168 Embedded islets were sliced by cryosectioning in 6 µM thin sections onto microscope slides.
169 Sections were rehydrated and washed with PBS. Antigen retrieval was performed with PBS
170 with 3% Triton for 15 min. After blocking non-specific antibody binding sites with background
171 sniper (Biocare Medical) for 11 min at room temperature, the sections were incubated at 4°C
172 with primary antibodies (Insulin/Glucagon/Somatostatin/ACSL4/TUNEL) diluted in PBS with
173 0,2%Triton, 2% BSA and 2% goat serum overnight. Sections were washed with PBS in 0.5%
174 Tween. Secondary antibodies together with 4′,6-diamidino-2-phenylindole (DAPI) were diluted
175 in PBS with 0,2%Triton,2% BSA, and 2% goat serum. After incubation for 1 h, the sections
176 were finally washed with PBS and 0.5% Tween. Immunofluorescence microscopy was
177 performed using Zeiss Axiovert200M with AxioCamMRc5.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 8. For comparison between groups, ordinary one-way-ANOVA ($p<0.05$) test with Tukey´s multiple comparison was used. Results were shown as mean ± SEM from N=X independent experiments.

185 **Results**

186 **RSL3 causes massive islet death in a dose-dependent manner and pretreatment with**
187 **Fer-1 can rescue islet viability and function**

188 We observed that ferroptosis induction with increasing concentrations of RSL3 resulted in a
189 significant reduction in islet viability (Fig. 1 A). At concentrations of 10 µM RSL3, islet viability
190 was reduced to 40.3% compared to 77.3% in the control group. Induction of ferroptosis with
191 40 µM RSL3 had a fatal effect on islet viability. In addition, TUNEL-staining was performed to
192 detect apoptotic cell death by labeling fragmented DNA and differentiate between apoptotic
193 and ferroptotic cell death (Fig. 5 A). Treatment with 10 µM RSL3 and Fer-1 alone and in
194 combination did not cause an increase in TUNEL positive cells compared to the control group.

195 The viability of islets challenged with 20 µM RSL3 could effectively be preserved by
196 pretreatment with 10 µM Fer-1 for 24 h (Fig. 1 B). Similar effects were observed regarding
197 islet function (Fig. 2). Pretreatment with Fer-1 increased functional potency compared to RSL3
198 induction alone. Interestingly, single treatment with Fer-1 led to a reduction of islet stimulation
199 Index (7.25 compared to 20.04 in the control group).

200 **Treatment with Fer-1 alone did not impact on islet survival, viability or islet architecture**

201 To determine whether ferroptotic cell death occurs as a common cell death mechanism in
202 cultured rodent islets, the culture time was prolonged to seven days. We observed that
203 treatment with Fer-1 alone over 7 days had no impact on islet viability (Fig. 3). In order to
204 prove whether the sustained islet viability is the result of a positive selection process during
205 islet culture, or whether Fer-1 has a direct effect on the survival of the islets in culture, we
206 examined cell survival over the period of seven days (quantification of IP; Fig. 3). Both groups
207 showed a steady decrease in IP/ml without significant difference.

208 With immunohistochemical analysis we could demonstrate that the prolonged treatment with
209 Fer-1 (Fig. 5 B) did not influence islet architecture.

210

211 **RSL3 and Fer-1 influence the intracellular iron, and zinc concentration of cultured**
212 **rodent islets**

213 In order to elucidate intracellular changes during ferroptosis, the intracellular iron and zinc
214 concentrations were measured. Culturing of the islets with the ferroptosis activator RSL3
215 increased the iron concentration to 0.88 ± 0.17 nmol/mg protein compared to 0.59 ± 0.05
216 nmol/mg protein (Fig. 4 A). Interestingly, pretreatment with the ferroptosis inhibitor Fer-1 could
217 significantly reverse this effect by almost 50% and resulted in an iron concentration of 0.42 ±
218 0.03 nmol/mg protein. Furthermore, Fer1 treatment showed a slight reduction of the iron
219 concentration in contrast to control. Several studies also describe ferroptosis induction by zinc,
220 so we decided to measure intracellular zinc concentration in addition to intracellular iron
221 concentration [12,13].

222 Similar to the effect on iron concentrations, a treatment of the islets with RSL3 alone
223 significantly increased the zinc concentration to 2.00 ± 0.30 nmol/mg protein in contrast to
224 control islets with 1.10 ± 0.03 nmol/mg protein. A pretreatment of RSL3 challenged islets with
225 Fer-1 could reduce the zinc concentration of the islets to 1.56 ± 0.05 nmol/mg protein (Fig. 4
226 A).

227

228 **Cell death induction by RSL3 causes an increase in biomarkers of lipid peroxidation in**
229 **ferroptosis**

230 Since there is no specific detection method of ferroptosis, we measured the malondialdehyde
231 (MDA) concentration as a surrogate parameter. MDA occurs as an endproduct of ferroptotic
232 lipid peroxidation (Fig. 4 B). Furthermore, the expression of ACSL4 (Fig.6) was determined as
233 a membrane enzyme that incorporates arachidonate lipid acids into the lipid membrane and
234 extremely sensitive to ferroptotic lipid peroxidation [2,3,14]. RSL3 challenged islets showed
235 an increased MDA concentration to 3.49 ± 0.79 nmol/mg compared to 1.63 ± 0.10 nmol/mg

236 protein in the control group. Pretreatment with Fer-1 (10 µM) was able to attenuate RSL3
237 induced MDA concentrations to 2.27 ± 0.06 nmol/mg protein.

238 Immunostainings were performed to visualize the expression of ACSL4 in the lipid membrane
239 of the islets (Fig. 6). All imaged islets showing a positive staining for ACSL4, independently of
240 the treatment group. However, 10 µM RSL3 enhanced ACSL4 protein level as indicated by a
241 strong and pronounced signal compared to control group. Whereas islets treated with 10 µM
242 Fer-1 alone or Fer-1 and RSL3 in combination led to reduced ACSL4 protein level compared
243 to the control group.

244

245 **Discussion**

246 The aim of the study was to better understand ferroptosis mechanisms in pancreatic islets and
247 thereby identify potential targets for protecting the islets from cell death in the context of
248 transplantation. Minimizing and preventing cell death processes in islet transplantation to
249 improve islet graft function has long been in the focus of islet research. The process of
250 preparation of the pancreas, followed by enzymatic and physical isolation of the islets, leads
251 to cellular stress with increasing radicals.[15,16] These could induce ferroptosis at any stage
252 of preparation and transplantation. We know that apoptotic cell death peaks 5 days after
253 culture and mainly affects beta cells. [17] For ferroptosis, it is still unclear at which stage it
254 occurs, but Desferroxamine (DFO) has been shown to improve islet and graft function.
255 Since ferroptosis requires available cellular iron, we and others have suggested that
256 ferroptosis plays at least some role for the functional limitations of islets after isolation and
257 transplantation. [15]
258 Along this line, Bruni *et al.* first discovered that pancreatic islets were sensitive to RSL3-
259 induced ferroptosis [6].
260 The current study addressed further, the significance of the ferroptosis pathway and the
261 potential of its inhibition on isolated pancreatic islets in a primary cell culture system. We
262 focused on the cellular changes caused by RSL3 and the subsequent induction of ferroptotic

263 cell death as well as the effects of Fer-1 on pancreatic islets. While the literature is replete on
264 the mechanism of action of RSL3 and Fer-1 on ferroptosis for several cell types, that for
265 pancreatic islets is just evolving [5,18]. The current study showed that pharmaceutical
266 induction of cell death by RSL3 causes an increase in typical ferroptosis-associated
267 biomarkers such as iron, MDA and the membrane protein ACSL4. The findings on the features
268 and the mechanism of ferroptosis in pancreatic islets are consistent with the observation in
269 other cell types [1,12,13]. Moreover, we showed that Fer-1 attenuated the toxic consequences
270 of RLS3 and thus protect the islets from ferroptosis. Moreover, TUNEL-staining with RSL3-
271 challenged islets did not show an increase in apoptosis thereby clearly differentiated between
272 apoptosis and ferroptosis cell death processes. Our results allow for better understanding the
273 effects of ferroptosis in islets and to differentiate more precisely between ferroptosis and other
274 cell death mechanisms such as apoptosis. In our *in vitro* setting, islets did not show significant
275 benefit due to the treatment with Fer-1 alone. Neither islet viability nor survival did improve
276 due to the treatment with Fer-1 alone. Hence, islet isolation and culture per se seem not to
277 substantially induce ferroptosis. However, islet function was indeed impaired due to treatment
278 with Fer-1. As Fer-1 accumulates in the membrane of the endoplasmic reticulum (ER) one
279 could speculate that Fer-1 is anchored in the ER membrane and thereby deteriorates islet
280 function [19] Anchoring Fer-1 in the ER membrane may reduce the function of the ER lipid
281 membrane, which causes a reduction of insulin maturation and misfolded proinsulin. This
282 could be one explanation of the reduced insulin stimulation capacity of Fer1 treated islets in
283 contrast to control islets.
284 However, in the setting of intraportal islet transplantation with known drawbacks associated
285 with hypoxic and inflammatory environmental conditions [20], it seems likely, that protective
286 mechanisms of ferroptosis mediated through Fer-1 may have a relevant positive effect.
287 Moreover, hepatocytes are the major iron storage in the human body, and this could further
288 affect the impact of ferroptosis (and its inhibition) on pancreatic islet grafts [21].
289
290

References

[1] Yang WS, Stockwell BR. Ferroptosis: Death by Lipid Peroxidation. Trends Cell Biol 2016; 26: 165–176.

[2] Yang WS, Kim KJ, Gaschler MM, et al. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proc Natl Acad Sci U S A 2016; 113: E4966–E4975.

[3] Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell 2017; 171: 273–285.

[4] Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: An iron-dependent form of nonapoptotic cell death. Cell 2012; 149: 1060–1072.

[5] Miotto G, Rossetto M, Di Paolo ML, et al. Insight into the mechanism of ferroptosis inhibition by ferrostatin-1. Redox Biol 2020; 28.

[6] Bruni A, Pepper AR, Pawlick RL, et al. Ferroptosis-inducing agents compromise in vitro human islet viability and function article. Cell Death Dis 2018; 9.

[7] Pathak V, Pathak NM, O'Neill CL, et al. Therapies for Type 1 Diabetes: Current Scenario and Future Perspectives. Clin Med Insights Endocrinol Diabetes 2019; 12

[8] Ludwig B. Islet Transplantation as a Treat- ment Option for Patients with Type-1 Diabe- tes Mellitus: Indication, Intention, and Outcome. Gastroenterologische und Hepatologische Erkrankungen 2014; 12: 5–9

[9] Warnock GL, Thompson DM, Meloche RM, et al. A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes. Transplantation 2008; 86: 1762–1766.

[10] McCall MD, Maciver AM, Kin T, et al. Caspase Inhibitor IDN6556 Facilitates Marginal Mass Islet Engraftment in a Porcine Islet Autotransplant Model. Transplantation 2012; 94.

[11] Ricordi C, Gray DWR, Hering BJ, et al. Islet isolation assessment in man and large animals. Acta Diabetol Lat 1990; 27.

[12] Palmer LD, Jordan AT, Maloney KN, et al. Zinc intoxication induces ferroptosis in A549 human lung cells. Metallomics 2019; 11: 982–993.

[13] Zhang C, Liu Z, Zhang Y, et al. "Iron free" zinc oxide nanoparticles with ion-leaking properties disrupt intracellular ROS and iron homeostasis to induce ferroptosis. Cell Death Dis 2020; 11.

[14] Doll S, Proneth B, Tyurina YY, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol 2017; 13: 91–98.

[15] Bruni A, Bornstein S, Linkermann A, et al. Regulated Cell Death Seen through the Lens of Islet Transplantation. Cell Transplant 2018; 27: 890–901

[16] Bottino R, Balamurugan AN, Tse H, et al. Response of Human Islets to Isolation Stress and the Effect of Antioxidant Treatment. 2004

[17] Paraskevas S, Maysinger D, Wang R, et al. Cell Loss in Isolated Human Islets Occurs by Apoptosis. Pancreas 2000; 20

[18] Seibt TM, Proneth B, Conrad M. Role of GPX4 in ferroptosis and its pharmacological implication. Free Radic Biol Med 2019; 133: 144–152

340 [19] Gaschler MM, Hu F, Feng H, et al. Determination of the Subcellular
341 Localization and Mechanism of Action of Ferrostatins in Suppressing
342 Ferroptosis. ACS Chem Biol 2018; 13: 1013–1020.
343 [20] Kanak MA, Takita M, Kunnathodi F, et al. Inflammatory Response in
344 Islet Transplantation. Int J Endocrinol 2014; 2014: 1–13.
345 [21] Anderson GJ, Frazer DM. Hepatic Iron Metabolism. Semin Liver Dis
346 2005; 25: 420–432.
347
348
349
350