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Mechanisms of browning and cell death in thermogenic adipocytes

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Table of content

List of abbreviations

AATBC	Apoptosis associated transcript in bladder cancer
ADOA	Autosomal dominant optic atrophy
AIFM2	Apoptosis inducting factor 2
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
CMT2A	Charcot-Marie-Tooth disease type 2A
CoQ10	Coenzyme Q10
DDI2	DNA Damage Inducible 1 Homolog 2
DNA	Deoxyribonucleic acid
DRP1	Dynamin-related protein 1
Fer-1	Ferrostatin-1
FSP1	Ferroptosis suppressor protein 1
GLP1R	Glucagon-like peptide 1 receptor
GPX4	Glutathione peroxidase 4
Lip-1	Liproxstatin-1
lncRNA	Long non-coding ribonucleic acid
MFN1/2	Mitofusin-1 and -2
NFE2L1	Nuclear factor erythroid-2 like bZIP transcription factor 1
NGLY1	N-glycanase 1
NST	Non-shivering thermogenesis
OPA1	Optic atrophy 1
RNA	Ribolnucleic acid
SLC7A11	Solute carrier family 7 member 11
SSMD	Sedaghatian-type spondylometaphyseal dysplasia
UCP1	Uncoupling Protein 1
UPS	Ubiquitin Proteasome System
WAT	White adipose tissue

List of publications

Original peer-reviewed publications that constitute this dissertation:

1. Giroud M*, **Kotschi S***, Kwon Y, Le Thuc O, Hoffmann A, Gil-Lozano M, Karbiener M, Higareda-Almaraz JC, Khani S, Tews D, Fischer-Posovszky P, Sun W, Dong H, Ghosh A, Wolfrum C, Wabitsch M, Virtanen KA, Bluher M, Nielsen S, Zeigerer A, Garcia-Caceres C, Scheideler M, Herzig S, Bartelt A. The obesity-linked human lncRNA AATBC stimulates mitochondrial function in adipocytes. **EMBO Rep. 2023**:e57600. (*equal contribution). (Impact Factor 2023: 6.5).

2. **Kotschi S**, Jung A, Willemsen N, Ofoghi A, Proneth B, Conrad M, Bartelt A. NFE2L1-mediated proteasome function protects from ferroptosis. **Mol Metab. 2022**;57:101436. (Impact Factor 2022: 8.1).

Further original peer-reviewed publications:

1. Ziegler KA, Ahles A, Dueck A, Esfandyari D, Pichler P, Weber K, **Kotschi S**, Bartelt A, Sinicina I, Graw M, Leonhardt H, Weckbach LT, Massberg S, Schifferer M, Simons M, Hoeher L, Luo J, Erturk A, Schiattarella GG, Sassi Y, Misgeld T, Engelhardt S. Immune-mediated denervation of the pineal gland underlies sleep disturbance in cardiac disease. **Science. 2023**;381(6655):285-90. (Impact Factor 2023: 44.7).

2. Sigmund AM, Winkler M, Engelmayer S, Kugelmann D, Egu DT, Steinert LS, Fuchs M, Hiermaier M, Radeva MY, Bayerbach FC, Butz E, **Kotschi S**, Hudemann C, Hertl M, Yeruva S, Schmidt E, Yazdi AS, Ghoreschi K, Vielmuth F, Waschke J. Apremilast prevents blistering in human epidermis and stabilizes keratinocyte adhesion in pemphigus. **Nat Commun. 2023**;14(1):116. (Impact Factor 2023: 14.7).

3. Muley C, **Kotschi S**, Bartelt A. Role of Ubiquilins for Brown Adipocyte Proteostasis and Thermogenesis. **Front Endocrinol (Lausanne). 2021**;12:739021. (Impact Factor 2021: 6.1).

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Reviews and commentaries:

1. Berndt C, Alborzinia H, Amen VS, Ayton S, Barayeu U, Bartelt A, Bayir H, Bebber CM, Birsoy K, Bottcher JP, Brabletz S, Brabletz T, Brown AR, Brune B, Bulli G, Bruneau A, Chen Q, DeNicola GM, Dick TP, Distefano A, Dixon SJ, Engler JB, Esser-von Bieren J, Fedorova M, Friedmann Angeli JP, Friese MA, Fuhrmann DC, Garcia-Saez AJ, Garbowicz K, Gotz M, Gu W, Hammerich L, Hassannia B, Jiang X, Jeridi A, Kang YP,

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2. Willemsen N*, **Kotschi S***, Bartelt A. Fire up the pyre: inosine thermogenic signaling for obesity therapy. **Signal Transduct Target Ther. 2022**;7(1):375. (*equal contribution). (Impact Factor 2022: 39.3).

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1. Jethwa C, Hoffmann A, **Kotschi S**, Caesar J, Kern M, Worthmann A, Schlein C, Khani S, Arús BA, Bruns OT, Ghosh A, Wolfrum C, Döring Y, Herzig S, Weber C, Blüher M, Widenmaier SB, Hotamışlıgil GS, Bartelt A. Control of cholesterol-induced adipocyte inflammation by the Nfe2l1-Atf3 pathway. **bioRxiv. 2024**:2024.07.22.604614.

2. Ofoghi A, **Kotschi S**, Lemmer IL, Haas DT, Willemsen N, Bayer B, Möller S, Haberecht-Müller S, Krüger E, Krahmer N, Bartelt A. Activating the NFE2L1-ubiquitin-proteasome system by DDI2 protects from ferroptosis. **bioRxiv. 2023**:2023.07.04.547652.

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4. Haumann F, Evangelakos I, Worthmann A, Liebold I, **Kotschi S**, Bischoff AT, Neuhofer CM, Schweizer M, Heine M, consortium tm, Büchner B, Klopstock T, Prehn C, Dyar KA, Prokisch H, Bosurgi L, Heeren J, Bartelt A, Kubisch C, Schlein C. Mitochondrial fatty acid synthesis is essential for coordinated energy transformation. **medRxiv. 2023**:2023.04.03.23288010.

Scientific presentations at international conferences:

May 2023	International Conference on Brown and Beige Fat – Organ Crosstalk, Signaling an Energetics, Hamburg, Germany *Adipocyte Nfe2l2 protects from adrenergic resistance during obesity* (poster presentation)
May 2023	FAPESP BAYLAT: Mitochondrial function and the regulation of energy metabolism, Riberão Preto, Brazil *Adipocyte Nfe2l2 protects from adrenergic resistance during obesity* (poster presentation)

April 2023	EMBO Workshop: Ferroptosis: When metabolism meets death, Seeon, Germany *Nfe2l1-mediated proteasomal activity protects brown adipose tissue from ferroptosis* (poster presentation)
March 2023	29th Annual Scandinavian Atherosclerosis Society Conference, Humlebæk, Denmark *Adipocyte Nfe2l2 protects from adrenergic resistance during obesity* (poster presentation)
November 2022	University of Toronto: Adipose Biology Seminar Series, online *Antioxidant Defense Mechanisms in Adipose Tissue* (oral presentation)
October 2022	Cold Spring Harbor Asia: Iron, Reactive Oxygen Species & Ferroptosis in Life, Death & Disease, Awaji, Japan *Nfe2l1-mediated proteasomal activity protects brown adipose tissue from ferroptosis* (poster presentation)
September 2022	European Lipoprotein Club: 45th Annual Scientific Meeting, Tutzing, Germany *The oxidative stress response in obesity is mediated by adipocyte Nfe2l2* (oral presentation)
August 2022	Keystone Symposium: Adipose Tissue and Metabolic Health, Whistler, Canada *Oxidative Stress and Obesity* (oral presentation) *The oxidative stress response in obesity is mediated by adipocyte Nfe2l2* (poster presentation)
May 2022	CPHBAT: The heterogeneity and plasticity of adipose tissue, Copenhagen, Denmark *The oxidative stress response in obesity is mediated by adipocyte Nfe2l2* (poster presentation)
December 2021	Danish Diabetes Academy: RNA Mechanisms & Therapeutics in Metabolic Disease, Copenhagen, Denmark *The obesity-linked human lncRNA AATBC regulates adipocyte plasticity by stimulating mitochondrial dynamics and respiration* (poster presentation)
April 2021	FöFoLe Statusseminar, online *NOXBAT: Nfe2l2 and oxidative stress in Brown Adipose Tissue* (oral presentation)
September 2020	European Lipoprotein Club: 43th Annual Scientific Meeting, Tutzing, Germany *Cold-activated mitohormesis in brown fat is mediated by Nfe2l2* (oral presentation)

Your contribution to the publications

1.1 Contribution to paper I

This is the first publication from the Bartelt Lab on the topic of ferroptosis. I conceptualized the topic of ferroptosis in brown adipose tissue myself and designed all experiments. All experiments were performed by myself or under my supervision, including statistical analysis. Coauthors assisted in specific techniques (native PAGE) and provided animal models. Data in Fig. 6 were collected by AB and analyzed by me. The manuscript was created and written by me with revisions from AB and scientific input from all coauthors. In addition, I helped to write a successful DFG proposal on this topic with AB.

1.2 Contribution to paper II

This manuscript is a joint work by the Herzig Lab and Bartelt Lab. The initial hypothesis was formulated by my co-first author, MG. MG was a postdoctoral researcher at the Herzig Lab and subsequently moved to Bartelt Lab before leaving in 2021. I joined the project in early 2021 and performed several experiments including ex vivo tissue analysis and in vitro experiments regarding mitochondrial function.

A detailed list of my contributions:

Figure 1: re-analysis of the sequencing data, including generation of 1F, Sup. 1C-F

Figure 2: generation of replicates for 2E-H, Sup. 2D-K

Figure 3: data generation of Sup. 3, Sup. 4MN; re-analysis 4D-O

Figure 4: data generation of 4E-H, M-P

Figure 5: data generation 5B, L-N, analysis of 5G-K

Figures 6, 7: data were analyzed by AH in collaboration with me

I wrote the manuscript together with MG, with revisions from SH and AB including input from all coauthors. All experiments during the revision period lasting two years were performed by me.

This work includes collaboration of international research groups for specific techniques and datasets. Especially in the final phase of the project, the communication between all collaborators was mediated by me.

2. Introduction

2.1 Thermogenic adipose tissue

Cardiovascular diseases are the most common cause of death in Germany[1]. Obesity is a major risk factor for cardiometabolic diseases and Type 2 Diabetes[2]. Therefore, adequate treatment of obesity is an unmet clinical need with the potential to not only extend the lifespan but also increase the quality of life of a large part of the population. The most effective treatment options today is still bariatric surgery, but in recent years pharmacological interventions based on the incretin hormone biology like Glucagon-like peptide 1 receptor (GLP1R)-agonists (semaglutide) have become promising therapeutic options[3,4]. However, these treatments have side-effects like nausea and vomiting. The activation of thermogenic adipose tissue has emerged in the past years as a promising target for the treatment of obesity and cardiovascular disease using natural inborn mechanisms that consequently should be devoid of side effects[5].

Adipose tissue in the human body can be subdivided into three major classes: white, beige, and brown adipose tissue. White adipose tissue (WAT) is the most abundant class in humans serving as a depot for lipids and the storage of spare energy. In contrast, the function of brown adipose tissue (BAT) is to burn calories to generate heat, a process known as non-shivering thermogenesis (NST). Beige adipose tissue is an intermediate form, which whilst developmental closely related to WAT is also thermogenically active. The highest prevalence of BAT, expressed as a fraction of bodyweight, is found in rodents, but also adipose tissue of hibernating mammals contains thermogenic potential[6,7]. The natural stimulus for BAT activation is cold exposure under the point of thermoneutrality. In most laboratory mice, thermoneutrality is achieved at approximately 30°C, leading to NST already at room temperature[8]. In humans this point is highly variable but is rarely fallen below for a longer time due to the advances of civilization like heating and clothing[9]. Therefore, BAT thermogenesis does not markedly contribute to total energy expenditure in adult humans. In contrast, a relatively large amount of BAT is found in neonates in the interscapular region but undergoes a drastic involution during infancy. In adults, remnants of BAT can still be found in the deep cervical, paravertebral, and perirenal region, especially during cold exposure or pharmacological stimulation[10]. Pharmacological activation of human BAT can be achieved by sympathomimetics, which target β-receptors on adipocytes, leading to the execution of the thermogenic program[11,12]. While this is showcasing that the remaining tissue is still functional, the therapeutical treatment with sympathomimetics is however unsuitable as cardiovascular side effects such as tachycardia and hypertension are to be expected. The prevalence of BAT has been shown to be positively correlated with cardiometabolic health in large human studies[13]. Activation of BAT in rodents has very strong positive effects on obesity, dyslipidemia and overall cardiometabolic health[14,15].

The unique color of BAT stems from its high content of iron-rich mitochondria. Unlike in most other tissues, BAT mitochondria contain a specialized protein called Uncoupling

Protein 1 (UCP1). UCP1 uncouples the proton gradient over the inner mitochondrial membrane, in which it resides. This leads to the catabolism of nutrients without the generation of adenosine triphosphate (ATP). Heat is produced as a byproduct of the futile enzymatic activity of the cell, which is used to regulate the core temperature of most mammals.

Next to the thermogenic activity of BAT in humans, the prevalence of beige adipose tissue in fat depots of humans has gained interest in recent years. Unlike BAT, which represents as developmental distinct entity to WAT, the term beige adipocyte describes a phenomenon of thermogenic activation of white adipocytes. These adipocytes undergo morphological changes from the large, univacuolar lipid droplet to a multivaculoar phenotype and increase both their mitochondrial content and their UCP1 expression, in a process known as browning[16].

2.2 Mitochondrial Dynamics

BAT is characterized by abundant mitochondria and high levels of respiration when fully activated. Mitochondria are regarded as the “powerhouse” of the cell, as they are the main site of cellular ATP production. The abundance of mitochondria can be regulated on the level of biogenesis and removal, a process known as mitophagy, which also serves as a quality control. Furthermore, mitochondria can undergo morphological changes to adapt to the changing demands of the cell by the processes of mitochondrial fission and fusion[17]. In the process of fusion, two or more mitochondria fuse into one bigger organelle, the reversal is referred to as fission. A magnitude of different stimuli can influence mitochondrial dynamics, however in the context of adipose tissue biology, nutrient availability and demand are next to the thermogenic state the main regulators of mitochondrial fission and fusion[18, 19]. Dynamin-related protein 1 (DRP1) is the main regulator of mitochondrial fission as it constricts the mitochondrion by forming a ring-like, oligomeric structure, which leads to the splitting of the organelle[20]. During mitochondrial fusion, two organelles are tethered to each other by mitofusins-1 and -2 (MFN 1/2) and merge their membranes[21,22]). The merging process is mediated by Optic atrophy 1 (OPA1)[23]. Genetic ablation of one of those molecules has been shown to shift mitochondrial dynamic to the opposite of their specific function. The full scope of effects of mitochondrial dynamics on the cell is not understood yet. It is however clear that it is a fine-tuned mechanism, which can be exemplified by the detrimental consequences of errors in dynamics. The neurological disorders Charcot-Marie-Tooth disease type 2A (CMT2A) or autosomal dominant optic atrophy (ADOA) are caused by mutations in the MFN2 and OPA1 gene respectively[24,25,26]. It has become apparent that neither fission nor fusion is always preferential to the other, but that both are necessary to keep the cell flexible to its challenges.

In adipocytes both fission and fusion seem to be necessary to execute the thermogenic program, with mitochondrial fission being associated with thermogenesis in humans[27,28].

On the other hand, fused mitochondria are also correlated with thermogenic activation and the abrogation of fusion events can attenuate thermogenic capacity[29,30]. When BAT is quiescent, for example when animals are adapted to thermoneutrality, mitochondrial content is diminished. This raises the question how cells deal under these conditions with excess iron liberated from the mitochondria.

2.3 Ferroptosis

Ferroptosis has emerged as a novel form of regulated cell death, yet it is distinct from the best described form of programmed cell death, apoptosis. It is mainly dependent on lipid peroxidation, a process which can be catalyzed by free intracellular iron by Fenton chemistry[31]. Whereas the term ferroptosis was only coined in 2012, the mechanisms protecting the cell against lipid peroxidation have been described in detail years before[32,33,34].

The enzyme Glutathione Peroxidase 4 (GPX4) has been proven to be the main anti-ferroptotic player, as its genetic deletion will cause ferroptotic cell death in various tissues[35]. GPX4 uses glutathione (GSH), a reductive agent highly abundant in the cytosol, to reduce and thereby detoxify lipid peroxides. Genetic deletion of the enzyme or pharmacological inhibition of GPX4 by the small molecule RSL3 causes ferroptosis in a dose-dependent manner, even though the precise molecular mechanism of RSL3 function has been disputed[32,36, 37]. The molecular mechanism by which the small molecule erastin causes ferroptosis, shows the dependency of GPX4 on GSH availability. Erastin inhibits the cysteine/glutamate antiporter system Xc- (encoded by solute carrier family 7 member 11 (SLC7A11)), thereby diminishing intracellular cysteine levels[32]. Cysteine levels become limiting for GSH synthesis, resulting in reduced GPX4 activity and ferroptosis. Next to the GPX4 system, coenzyme Q10 (CoQ10) can be used as an antioxidant in membranes. Ferroptosis suppressor protein 1 (FSP1, formerly also known as apoptosis inducing factor 2 (AIFM2)) can utilize CoQ10 to reduce lipid peroxides[38, 39]. Direct pharmacological inhibitors of FSP1 have only been described recently, at the time I conducted the experiments for our study, FIN56 was the gold standard to test this anti-ferroptotic axis[40, 41]. FIN56 activates squalene synthase, thereby redirecting the mevalonate pathway towards cholesterol synthesis and away from CoQ10 synthesis, reducing CoQ10 availability and causing ferroptosis[42]. Pharmacological inhibition of ferroptosis can be achieved by lipid antioxidants, which chemically defuse lipid peroxides. Next to the naturally occurring Vitamin E, the small molecules ferrostatin-1 and liproxstatin-1 have been shown to be potent inhibitors of ferroptosis[43].

The clinical relevance of ferroptosis is poorly understood, but it has been implicated both as a risk factor, e.g. in neurodegenerative diseases an ischemia-reperfusion injury, and as potential therapeutic target for tumor therapy[44]. Sedaghatian-type spondylometaphyseal dysplasia (SSMD) is an autosomal-recessive rare genetic disorder caused by mutations in the *GPX4* gene, leading to a reduced GPX4 activity[45]. It presents with skeletal

malformations, as wells as cardiovascular and neurological abnormalities leading to a severely shortened life expectancy of a few days to a few years, depending on the residual activity of GPX4[46].

As brown adipocytes largely consist of iron-rich mitochondria and lipids, they offer in theory a ferroptosis-prone environment. At the time of publication, no conclusive studies were available on the role of ferroptosis in adipose tissue or thermogenesis. Interestingly, it was previously shown that degenerated BAT due to loss of proteostasis is characterized by high levels of labile iron[47].

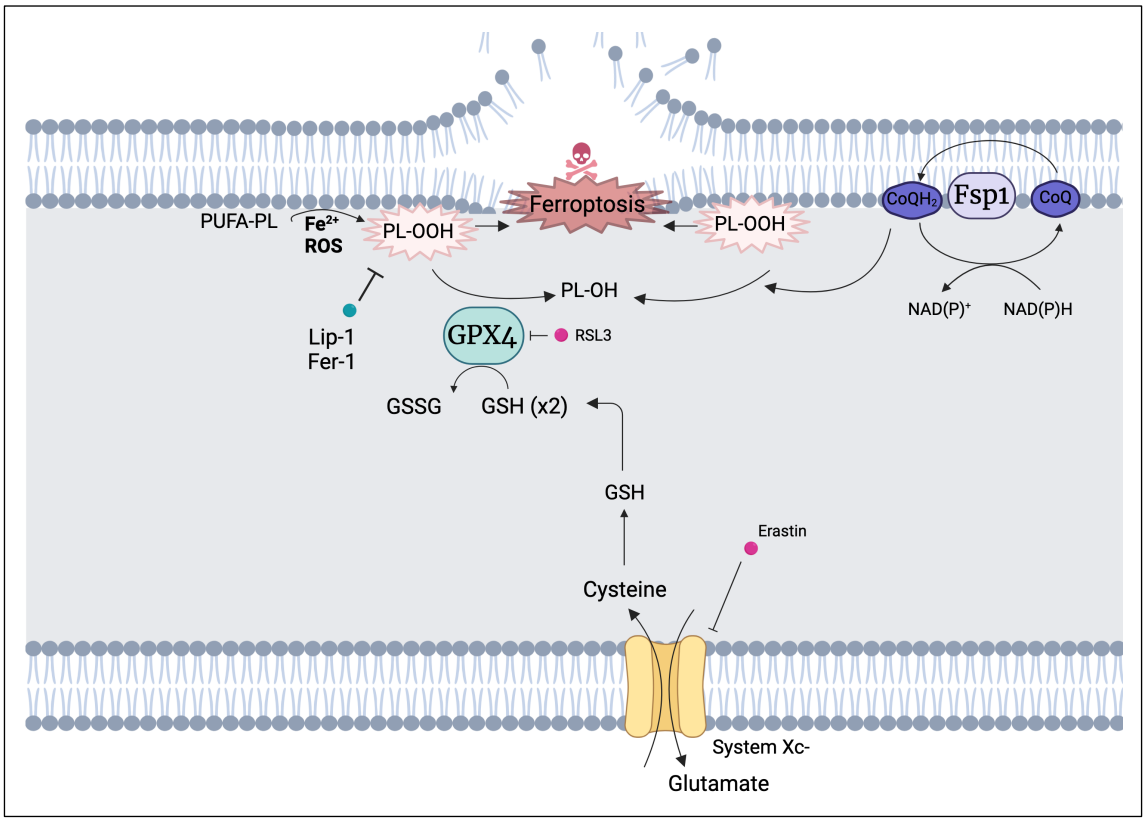

Figure 1: Mechanisms of ferroptosis

Ferroptosis describes cell death by peroxidation of polyunsaturated fatty acids in phospholipids (PUFA-PL). This process is mediated by free iron (Fe2+) and reactive oxygen species (ROS). Reduction of the peroxides (PL-OOH -> PL-OH) is catalyzed by GPX4 by oxidation of glutathione or by FSP1 which uses CoQ10 as a substrate. The glutathione pool is limited by cysteine abundance which is regulated by the cysteine/glutamate antiporter System Xc-. Ferroptosis can be pharmacologically inhibited by lipid antioxidants such as ferrostatin-1 (Fer-1) and liproxstatin-1 (Lip-1). Amongst the inducers of ferroptosis are RSL3, which is a GPX4-inhibitor, and erastin which blocks cysteine import via System Xc-. Created with Biorender.com.

2.4 Ubiquitin Proteasome System (UPS)

The UPS is essential for the cell to maintain homeostasis between protein synthesis and protein degradation, a process known as proteostasis. To facilitate specific targeted removal, unwanted proteins are tagged with a ubiquitin chain by E3 ligases and degraded by the proteasome into small peptides[48]. The proteasome is a large molecular complex made up of a 20S core and a 19S regulatory unit, however, the constitution of the proteasome can be adapted to cellular conditions and stress (e.g. immunoproteasome)[49,50]. Further adaptation of proteasomal activity can be achieved by regulating the number of proteasomes in a cell. A key regulatory component for this is the transcription factor nuclear factor erythroid-2 like bZIP transcription factor 1 (NFE2L1, also known as NRF1/TCF11). NFE2L1 is translated into the membrane of the endoplasmic reticulum, undergoes cleavage by DNA Damage Inducible 1 Homolog 2 (DDI2) and deglycosylation by N-glycanase 1 (NGLY1), and is released into the cytosol where it is immediately degraded by the proteasome. However, when proteasomal activity is compromised or the rate of ubquitination is increased, NFE2L1 escapes degradation and translocates to the nucleus where it positively regulates the expression of proteasomal subunits[51].

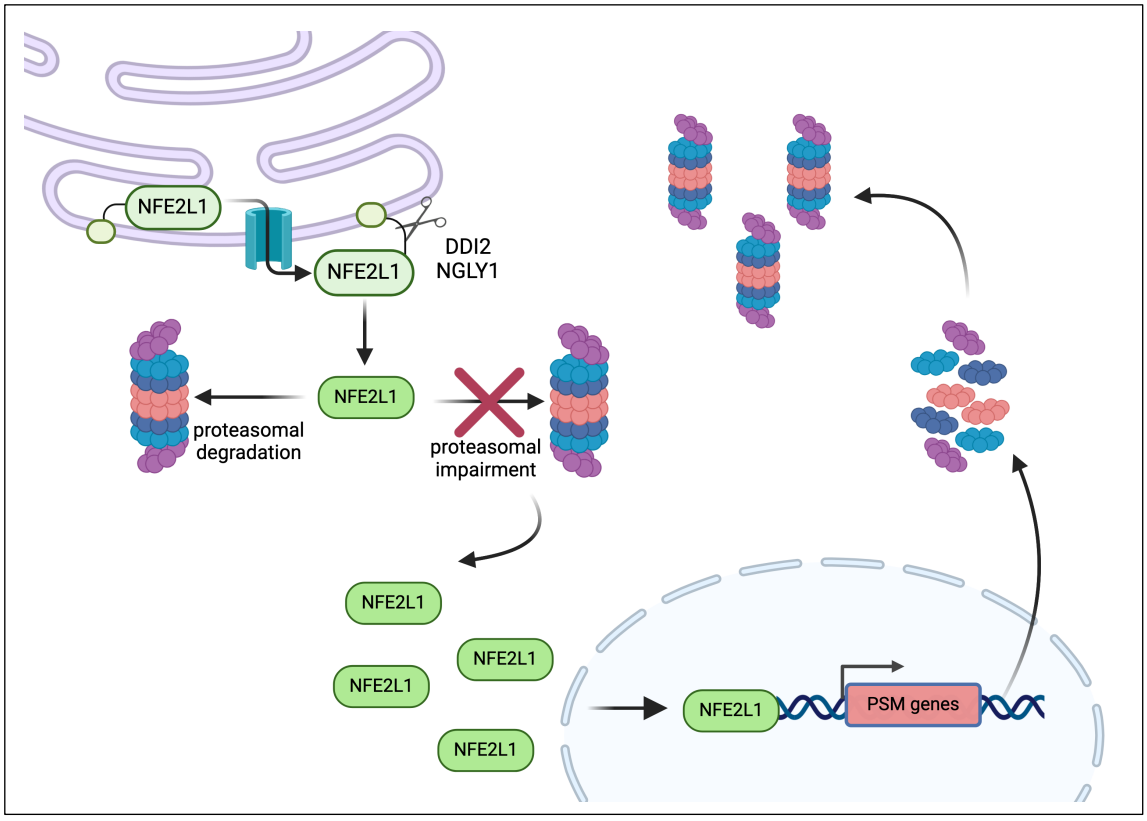

Figure 2: NFE2L1 as master regulator of the proteasome
NFE2L1 gets translated in the endoplasmic reticulum, processed by DDI2 and NGLY1 and is released into the cytoplasm. During homeostasis, NFE2L1 gets constantly degraded by the proteasome. If proteasomal activity is impaired, NFE2L1 gets translocated to the nucleus where it acts as a transcription factor for proteasomal subunit genes. This leads to an enhanced assembly of proteasomes to regain the equilibrium. Created with Biorender.com. Adapted from [52].

Nfe2l1 has been shown to be essential for the remodeling of BAT during cold adaptation, as this process is dependent on an adaptive increase of proteasomal activity. Genetic depletion renders BAT incapable of heat production and phenocopies the inactive state of BAT during thermoneutrality[53,47].

In paper 1, we elucidate the role of the UPS in ferroptosis[54]. As proteasomal activation has been shown to be highly relevant for BAT adaptation, we used brown adipocytes as a model. We observed in multiple in vivo and in vitro models a drastic reduction of proteasomal activity during the early stages of ferroptosis where cell death does not occur yet. Furthermore, we were able to expand this observation to other cell types, such as the human fibrosarcoma cell line HT-1080 and primary fibroblasts from a patient with SSMD. As NFE2L1 is a master regulator of the proteasome, we investigated if ferroptosis is modulated by NFE2L1. Induction of ferroptosis lead to a strong increase of NFE2L1 activity and genetic inhibition of NFE2L1 made cells more sensitive to ferroptosis.

Our results on the role of NFE2L1 in ferroptosis were largely replicated by another study published shortly after our manuscript[55].

2.5 Long non-coding ribonucleic acid (lncRNA)

The first discovered function of RNA[56,57] was as a messenger from genomic deoxyribonucleic acid (DNA) to ribosomes for protein synthesis. Next to this coding form of RNA, in later years a multitude of RNA species that are not encoding proteins have been discovered, amongst them ribosomal RNA, transfer RNAs, and micro RNAs. Even though most of the research is conducted on mRNA, non-coding RNA are with 98% of all transcripts the more abundant species[58]. Among those species are long non-coding RNA, defined as molecules longer than 200 nucleotides, even though their potential for encoding peptides or short-lived proteins has been topic of discussion[59, 60]. The conservation of lncRNA between species is limited, therefore they are hypothesized to be a large contributor to inter-species variability[60]. lncRNA are implicated in several cellular processes such as the regulation of transcription and translation, but the knowledge about exact mechanisms is limited and large number of interaction partners have been identified[61].

The research about lncRNA in metabolism and adipocyte biology is limited, but multiple candidates have been implied in major pathways of browning[62, 63,64]. Most of those studies were conducted in mouse models. To address distinct human adipose tissue physiology, in paper 2 we are however characterizing a human-specific lncRNA[65].

Using a combination of transcriptional profiles of human thermogenic adipose tissues, we identified the lncRNA Apoptosis associated transcript in bladder cancer (AATBC) to be consistently upregulated in thermogenic conditions. Modulation of AATBC levels in models of human thermogenic adipocytes was positively correlated with thermogenic markers, mitochondrial activity, and mitochondrial fission. In both a mouse model with exogeneous expression of AATBC and humans, AATBC was correlated with lower leptin levels and higher markers of metabolic health.

3. Summary

Obesity is a major risk factor for cardiovascular diseases, the most common cause of death in Germany. Studies in rodents have shown that the activation of BAT is a promising way to fight obesity and cardiovascular disease utilizing the inborn mechanism of NST. Among the many unanswered questions about BAT biology are the mechanisms of involution of the tissue during infancy and how to translate findings in rodents to humans.

In the first publication I investigated the role of the recently discovered cell death mechanism “ferroptosis” in brown adipose tissue. I discovered that ferroptosis compromises the UPS in brown adipocytes, a pathway that has been shown to be essential for brown adipose tissue homeostasis. NFE2L1 acts as a master regulator of the UPS and can restore proteasomal activity during proteotoxic stress. Surprisingly, NFE2L1 is strongly activated by inducers of ferroptosis. Genetic depletion of NFE2L1 lead to increased sensitivity towards ferroptosis in brown adipocytes and tumor cell lines. On top of that patient-derived fibroblasts carrying a mutation in the anti-ferroptotic enzyme GPX4 replicated this phenotype, proving the relevance of NFE2L1 in ferroptosis protection in multiple models. Mice lacking Nfe2l1 in BAT present whitening of the tissue with a loss of thermogenic capacity. Signatures of ferroptosis were also observed in brown adipose tissue of mice lacking Nfe2l1, hinting at a possible role of ferroptosis in BAT involution. In summary, we discovered a novel anti-ferroptotic mechanism in brown adipocytes, in which NFE2L1 promotes proteasomal activity to prevent ferroptosis.

Adipose tissue research is usually performed using the mouse as a model organism. Due to the vastly reduced prevalence of BAT in humans it is necessary to find mechanisms in thermogenic adipose tissue that are functional in humans.

In the second study we found the human specific long non-coding RNA AATBC to be a modulator of thermogenesis. Using transcriptomic analysis from human adipose tissue and cell lines we found AATBC to be enriched in thermogenic conditions. Modulating the expression levels of AATBC revealed a positive correlation with markers of thermogenesis and mitochondrial activity. As mitochondrial abundance was unaltered, we observed changes in mitochondrial dynamics with AATBC promoting mitochondrial fission, which is associated with thermogenesis. Since AATBC is only expressed in humans, we used virus-mediated overexpression in mouse adipose tissue to study the effects of the lncRNA in vivo. We found leptin levels to be suppressed in animals expressing AATBC, which could also be observed in independent human cohorts. In humans, AATBC expression is furthermore negatively correlated with bodyweight and body mass index, and positively correlated with markers of adipose tissue browning. In conclusion, we describe a novel human-specific lncRNA that promotes adipose tissue browning my shifting mitochondrial dynamics to fission-like phenotype.

4. Zusammenfassung

Adipositas ist ein Risikofaktor für kardiovaskuläre Erkrankungen, die häufigste Todesursache in Deutschland. Studien im Tiermodell haben gezeigt, dass die Aktivierung von braunem Fettgewebe (BAT) eine vielversprechende Methode zur Bekämpfung von Adipositas und kardiovaskulären Erkrankungen darstellt, indem sie den angeborenen Mechanismus der zitterfreien Thermogenese nutzt. Zu den vielen unbeantworteten Fragen gehören die Mechanismen der BAT-Involution des Gewebes im Säuglingsalter und wie sich Erkenntnisse aus dem Tiermodell auf den Menschen übertragen lassen. In der ersten Publikation untersuchte ich die Rolle des kürzlich entdeckten Zelltodmechanismus "Ferroptose" im braunen Fettgewebe. Ich stellte fest, dass Ferroptose das UPS in braunen Adipozyten beeinträchtigt, welches für die Homöostase des braunen Fettgewebes als wesentlich gilt. NFE2L1 fungiert als Hauptregulator des UPS und kann die proteasomale Aktivität während proteotoxischem Stress wiederherstellen. Überraschenderweise lässt sich bei Induktion von Ferroptose eine substanzielle Aktivierung von NFFE2L1 feststellen. Die genetische Depletion von NFE2L1 führte zu erhöhter Empfindlichkeit gegenüber Ferroptose in braunen Adipozyten und Tumorzelllinien. Dieses Phänomen konnte in Fibroblasten eines Patienten mit einer Mutation im anti-ferroptotischen Enzym GPX4 repliziert werden, was die Relevanz von NFE2L1 im Schutz vor Ferroptose in mehreren Modellen beweist. Mäuse ohne Nfe2l1 im BAT zeigen eine Aufhellung des Gewebes und einen Verlust der thermogenen Kapazität. In diesen lassen sich ebenfalls Anzeichen von Ferroptose beobachten, was auf eine mögliche Rolle der Ferroptose bei der Involution von BAT hindeutet. Zusammenfassend haben wir einen neuartigen anti-ferroptotischen Mechanismus in braunen Adipozyten entdeckt, bei dem NFE2L1 durch Aufrechterhaltung der proteasomalen Aktivität Ferroptose verhindert.Die Forschung an Fettgewebe wird üblicherweise am Mausmodell durchgeführt. Aufgrund der stark reduzierten Prävalenz von BAT beim Menschen ist es notwendig, Mechanismen im thermogenen Fettgewebe zu finden, die beim Menschen funktional sind. In der zweiten Studie fanden wir, dass die, spezifisch im Menschen exprimierte, long non-coding RNA AATBC ein Modulator der Thermogenese ist. Mittels Transkriptomanalyse von menschlichem Fettgewebe und Zelllinien stellten wir fest, dass AATBC vermehrt unter thermogenen Bedingungen vorliegt. Durch Modulation der Expression von AATBC zeigte eine positive Korrelation mit Markern der Thermogenese und mitochondrialer Aktivität. Da die Anzahl an Mitochondrien unverändert war, untersuchten wir Veränderungen in der mitochondrialen Dynamik. AATBC förderte die mitochondriale *fission*, die mit Thermogenese assoziiert ist. Da AATBC nur im Menschen exprimiert ist, nutzten wir virusvermittelte Überexpression im Mausfettgewebe, um die Effekte der lncRNA in vivo zu untersuchen. Wir stellten fest, dass die Leptin-Spiegel in Tieren mit AATBC verringert waren, was wir auch in unabhängigen Patientenkohorten beobachten konnten. In klinischen Studien korreliert die AATBC-Expression negativ mit dem Körpergewicht und Body-Mass-Index und positiv mit Markern der Thermogenese des Fettgewebes. Zusammenfassend beschreiben wir eine neuartige, menschenspezifische lncRNA, die die Thermogenese fördert, indem sie die mitochondriale Dynamik hin zu einem *fission*-ähnlichen Phänotyp verschiebt.

5. Paper I

Kotschi S, Jung A, Willemsen N, Ofoghi A, Proneth B, Conrad M, Bartelt A.

NFE2L1-mediated proteasome function protects from ferroptosis.

Mol Metab. 2022;57:101436. (Impact Factor 2002: 8.1)

6. Paper II

Giroud M*, **Kotschi S***, Kwon Y, Le Thuc O, Hoffmann A, Gil-Lozano M, Karbiener M, Higareda-Almaraz JC, Khani S, Tews D, Fischer-Posovszky P, Sun W, Dong H, Ghosh A, Wolfrum C, Wabitsch M, Virtanen KA, Bluher M, Nielsen S, Zeigerer A, Garcia-Caceres C, Scheideler M, Herzig S, Bartelt A. (*equal contribution)

The obesity-linked human lncRNA AATBC stimulates mitochondrial function in adipocytes.

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