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AKR1C1 protect mature oligodendrocytes from ferroptotic cell death

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Contents

Abstract

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Ferroptosis is a form of iron-dependent programmed cell death, regulated by specific pathways and characterized by genetic, biochemical, and morphological features that distinguish it from other cell death mechanisms, such as apoptosis and necrosis. Ferroptosis has been implicated in various neuroinflammatory disorders, including Parkinson's and Alzheimer's diseases, and may also contribute to oligodendrocyte degeneration during the onset and progression of Multiple Sclerosis.

Oligodendrocytes (OLs) are specialized neural cells responsible for forming myelin sheath that insulates neurones and ensures efficient signal transmission.

To investigate the potential connection between ferroptosis and oligodendrocyte loss and demyelination, we analyzed the induction and execution of ferroptosis at the molecular level in the human MO3.13 oligodendrocyte cell line (OLs). Interestingly, our findings revealed that mature OLs exhibit resistance to ferroptosis, regardless of the inducing stimulus, in contrast to their immature counterparts.

The ongoing search for molecular players involved in ferroptosis resistance led us to investigate the role of Aldo keto-reductase family 1 member C1 (AKR1C1) in mature OLs ferroptotic death. AKR1C1 is detoxifying enzymes known to contribute to ferroptosis resistance.

Recent findings suggest that the enzyme Aldo-keto reductase family 1 member C1 (AKR1C1) may play a critical protective role in ferroptotic resistance. However, its potential protective mechanisms within mature OLs against ferroptosis is not known.

In this study, we observed that high expression levels of AKR1C1 in mature OLs significantly reduced the ferroptotic cell death. These results can offer an interesting starting point to outline a new potential anti-ferroptotic pathway and a new approach for the treatment of neurodegenerative disease which often do not respond to conventional therapy.

1. introduction

1.1 ferroptosis

Cell death is a crucial process for normal development, homeostasis, and the prevention of hyperproliferative diseases such as cancer. The most common form of cell death in mammalian cells is mediated by the activation of caspase-dependent apoptosis. However, recent research has identified several regulated non-apoptotic cell death pathways that are activated in specific disease states[1].

In 2013, in the Stockwell laboratory a new compound, erastin, was discovered which had a selectively lethal effect on RAS mutated cancer cells, but the pattern of cell death was different from what had been described before [2]. Subsequently, a RAS-selective lethal small molecule3 (RSL3) has been identified which was able to cause the same pattern of cell death[3].

The new form of cell death named ferroptosis which is identified as a nonapoptotic forms of cell death may facilitate the selective elimination of some tumor cells or be activated in specific pathological states. This form of cell death was named ferroptosis due to the key role of iron.

Ferroptosis is morphologically, biochemically, and genetically distinct from apoptosis, necrosis, and autophagy[1].

Since it lacks the typical morphological characteristics of apoptosis, such as nuclear fragmentation, mitochondrial cytochrome c release, and generation of apoptotic bodies. In fact, cells are usually rounded up and detached in response to erastin; they exhibit changed mitochondrial morphology, which appear smaller with increased membrane densities and reduced cristae; there is no condensation of chromatin but, however, there is an increased level of intracellular reactive oxygen species (ROS), and cell death is completely inhibited by iron chelation or genetic inhibition of cellular iron uptake.[4,5]

Ferroptosis is characterized by the production and accumulation of lipid peroxides (Lipid-ROS), which are a key step in this newly identified form of cell death. The exact mechanisms of lipid-ROS toxicity are still debated. Recent findings suggest that it may

involve their integration into phospholipids (mainly PE-OOH) and translocation into the cell membrane, thereby, compromising membrane protein activity and/or membrane physiology and structure[6].

The production of lipid peroxides within cells is a consequence of various factors such as iron metabolism, lipoxygenase activity and ROS production[7].

1.1.1 Iron metabolic pathway and ferroptosis

Iron metabolism is recognized as a central mediator of ferroptosis since iron is essential for lipid peroxidation. In fact, supplementing the growth medium with a bioavailable form of iron, but not other divalent metals, accelerates erastin-induced ferroptosis[1]. Iron overload induces membrane lipid peroxidation by facilitating the catalytic breakdown of $H2O_2$ into hydroxyl radicals and superoxide anions, two highly reactive oxygen species (ROS)**,** through chemical reactions known as the Fenton and Haber-Weiss reactions. Iron acts as a cofactor in both inorganic chemical reactions and enzymatic reactions, which are responsible for the iron-dependent accumulation of lipid peroxides (Lipid-ROS) during ferroptosis execution[8].

An example of an enzymatic reaction is the oxidation of polyunsaturated fatty acids (PUFAs) into lipid hydroperoxides mediated by lipoxygenases (LOX). LOX, a family of non-heme iron-containing enzymes, plays a crucial role in ferroptosis by promoting lipid peroxidation. The sensitivity to lipid peroxidation and ferroptosis depends on iron uptake, storage, and export[3].

The internalization of iron in cells occurs through the membrane protein transferrin receptor 1 (TFR1), which facilitates the uptake of extracellular ferric iron (Fe3+) carried by transferrin (TF) into the endosome. In the endosome, (Fe3+) is reduced to ferrous iron (Fe 2+) by the ferrireductase STEAP3 (six-transmembrane epithelial antigen of the protease 3). Fe2+ can then be released from the endosome into the cytoplasm by the divalent metal transporter 1 (DMT1). In the cytoplasm, iron can bind to ferritin, a storage protein.

Conversely, intracellular iron can be exported out of the cell through the membrane protein ferroportin (FPN). (Figure 1) [9].

Increased iron uptake (due to higher TFR1 expression) and reduced iron storage (due to lower FTL and FTH1 expression) enhance the ferroptosis sensitivity of cells, in contrast to ferroptosis-resistant cells[10].

There are factors that reduce the ferroptosis sensitivity of cells. Iron chelators, such as Deferoxamine (DFO) and Ciclopiroxolamine (cpx), protect cells from ferroptosis by limiting iron availability and preventing lipid peroxidation.

Additionally, downregulation of IREB2 leads to an increase in iron metabolism-associated gene expression (e.g., TFR.FTH1, and FTL) and reduces erastin-stimulated ferroptosis. Thus, IREB2 encodes a master transcription factor that regulates iron metabolism[1].

Ferritinophagy is the autophagic degradation of ferritin, which stimulates ferroptosis by increasing labile iron levels. Nuclear receptor coactivator 4 (NCOA4) is a cargo receptor responsible for autophagy-dependent ferritin degradation. It binds to ferritin and transports it to phagophores, and subsequently to autophagolysosomes, where ferritin is degraded[11].

The levels of NCOA4 are regulated by intracellular iron. When intracellular iron levels are high, NCOA4 undergoes degradation by the proteasome, a process mediated by HERC2. Consequently, there is a negative correlation between HERC2 activity and ferroptosis induction. Moreover, impaired ferritinophagy may lead to increased IREB2 activity, which subsequently upregulates transcription factors as a feedback mechanism[12].

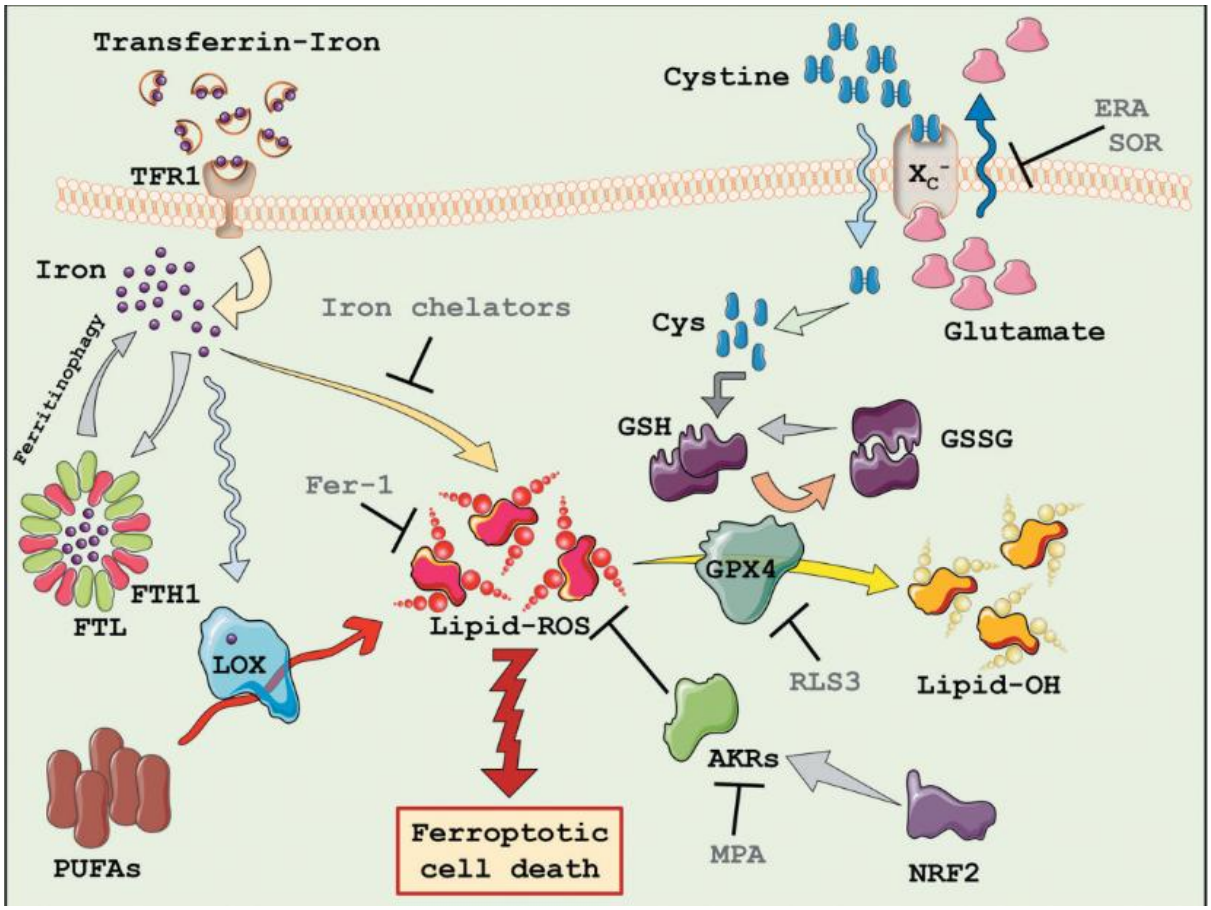

Figure 1: Iron plays a central role in ferroptosis by promoting lipid peroxidation. Trasferrine receptor 1 (TFR1) mediates iron uptake into the cell, while ferritin stores excess iron. The degradation of ferritin through ferritinophagy releases free iron, increasing ferroptosis sensitivity. Free iron catalyzes the Fenton reaction, generating reactive oxygen species (ROS) that drive lipid peroxidation, ultimately promoting ferroptosis[13]

1.1.2 lipid peroxidation metabolism

Lipids containing Polyunsaturated fatty acids (PUFAs) with labile bis-allylic hydrogen atoms are highly prone to lipid peroxidation, and their oxidized forms play a central role in ferroptosis[14]. Three key enzymes mediate lipid oxidation: Cyclooxygenases (COXs), Cytochrome P450 (CYPs), and Lipoxygenases (LOXs). Among these, LOXs play a crucial role by catalyzing the deoxygenation of PUFAs in cell membranes, leading to formation of lipid hydroperoxides and promoting ferroptosis[15].

The disruption of GPX4 and the increased production of ROS lead to alterations in the expression of genes associated with lipid metabolism, such as ACSL4 and PE, thereby promoting cell death[16]. ACSL4 plays a crucial role in lipid peroxidation by catalyzing the esterification of arachidonic acid (AA) into PE. Subsequently, LOX-15 facilitates the oxidation of AA-PE, generating lipid peroxides that drive ferroptosis[17].

Free lipid radical formation occurs as a consequence of a chain reaction initiated by a free radical that extracts an electron from PUFAs. The resulting lipid radical then reacts with oxygen to produce a peroxyl radical, which transforms polyunsaturated fatty acids into lipid hydroperoxides. Lipid ROS molecules are characterized by their instability and disruptive effect on the cell membrane[18].

Ferroptosis is triggered by an imbalance between lipid hydroperoxide (LOOH) detoxification and iron-dependent L-ROS accumulation[19].

Downstream accumulation of lipid peroxides represents a key step mediating the execution of ferroptosis. Antioxidant agents can block ferroptosis by inhibiting the lipid peroxidation pathway. These agents act as potent peroxyl radical scavenger with high affinity for unpaired electrons breaking a cascade of chain reactions in the lipid peroxidation of membranes. Additionally, ferroptosis suppressor protein 1(FSP1) confers protection against ferroptosis induced by GPX4 deletion and cooperates with GPX4 and glutathion to suppress phospholipid peroxidation and ferroptosis. Moreover, lipoxygenases inhibitors can supress LOX activity to rescue cells from ferroptosis.[13]

1.1.3 key regulators of ferroptosis: system Xc- and GPX4

Cystine-glutamate antiporter (system Xc-) is a heterodimeric integral cell membrane antiporter that uptake cystine from the extracellular space exchanging one molecule of cystine with one molecule of glutamate. It comprises two subunits: SLC7A11 which passes the membrane 12 time and bound to SLC3A2 via disulfide bridge that passes one time through membrane[20]. Cystine, the oxidized form of cysteine, is converted to cysteine which is a precursor for GSH synthesis. The expression of system XC- is regulated by the nuclear factor erythroid 2-related factor 2 (NRF2), which enhances its transcription under oxidative stress[21]. Furthermore, p53 can also modulate the activity of the system XC-, downregulating the expression of SLC7A11, thus resulting in a reduction in cell antioxidant capacity, accumulation of lipid-ROS, thus inducing the ferroptotic process[22]. The intake of cystine is associated to the release of glutamate, which is strictly controlled since high extracellular glutamate concentrations are neurotoxic[23]. The inhibition of system Xc- deprives the cellular cystine, making it unavailable for GSH synthesis. Depletion of GSH leads to the inhibition of Glutathione peroxidase 4 activity and resulting in accumulation of lipid ROS, protein or membrane damage, ultimately ferroptotic cell death (figure 2)[24].

Glutathione peroxidases (GPX) are a family of antioxidant enzymes responsible for reducing per-oxidized molecules into lower reactive alcohols. Specifically, GPX4 is the only GPX known to catalyze the reduction of lipid hydroperoxides (Lipid-ROS), essential for life, since its knockout causes embryonic lethality[25,26]. GPX4 converts glutathione into oxidized glutathione (GSSG) concomitantly reducing the cytotoxic lipid peroxides (LOOH) to the corresponding alcohol (L-OH). RSL3 directly acts on GPX4 inhibiting its activity, thus promoting lipid-ROS accumulation, decreased GPX4 expression, and inducing ferroptosis[27]. While GPX4 overexpression confers resistance to ferroptosis induced by RSL3[28]. To note, GPX4 overexpression is also associated with poor prognosis in patients with diffuse large B-cell lymphoma.[29]

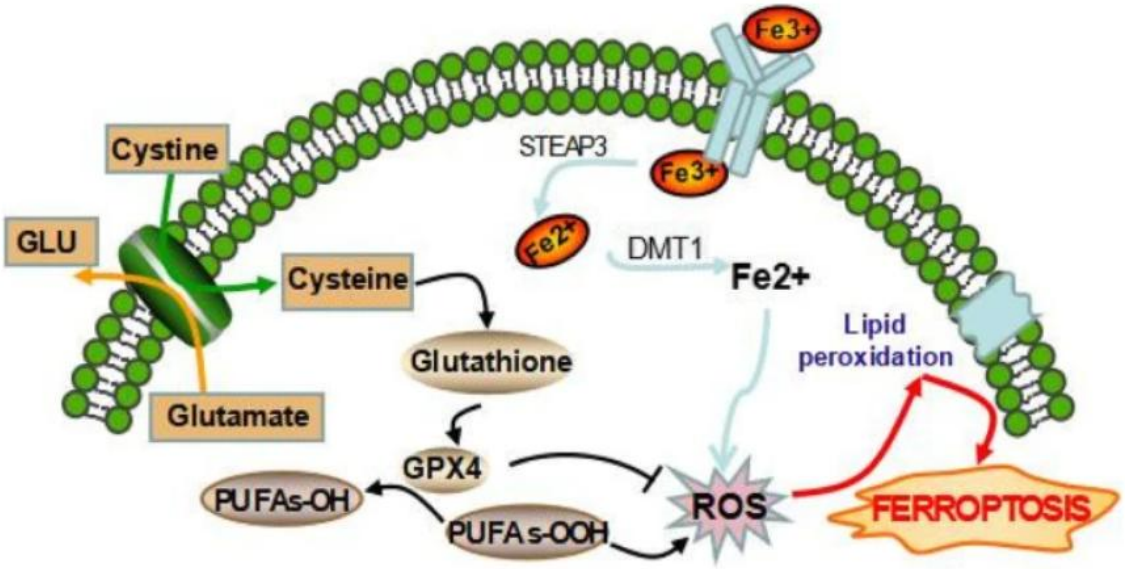

Figure 2: Mechanism of ferroptotic cell death. System xc- transports intracellular Glutamate to the extracellular space and extracellular Cys2 into the cell, which is then transformed into Cys for GSH synthesis. GPX4 reduces the endogenous neutralization of PUFAs-OOH to PUFAs-OH, ultimately reducing ROS accumulation. The iron excess is the basis for ferroptosis execution. Circulated iron was combined with transferrin in the form of Fe^{3+}, and then it entered into cells by TFR1. Iron in Fe^{3+} form was deoxidized to iron in Fe^{2+} by iron oxide reductase STEAP3. Ultimately, Fe^{2+} was released into a labile iron pool in the cytoplasm from the endosome mediated by DMT1[30].

1.1.4 Antioxidant defense in ferroptotic cell death

The imbalance between injury and defense signals leads to cell death. Classic ferroptosis inducers are inhibitors of the antioxidants system Xc-glutathione (GSH)-glutathione peroxidase 4 (GPX4) axis. In addition, a large number of anti-ferroptosis mediators are transcriptionally regulated by the nuclear factor, erythroid 2-like2 (NFE2L2, known as NRF2) pathway[31]. In absence of glutathione, cells initiate a complex of defense system, allowing their survival and cytoprotection. Upon chemical GSH depletion, NRF2, the antioxidant master transcription factor, can be activated. Newly evidence suggest that ER stress (with unfolded protein accumulation) can activate the NRF2-KEAP1 signaling pathway in a PERK-dependent manner. Once activated, PERK induces modifications of specific KEAP1 cystine residues leading to its conformational changes and resulting in the release of NRF2 from KEAP1. After release, NRF2 translocates from cytosol into the nucleus, binds to the Antioxidant Responsive Elements (AREs) and enhances the expression of antioxidant factors (quinone oxidoreductase 1, NQO), molecular chaperons, tumor suppression (such as p53) and intracellular detoxification genes (Glutathione S-transferase, GST; Aldo keto reductase, AKR; and Heme oxygenase, HO-1)[32,33].

Among all genes with antioxidant activity, particularly important is FSP1 which is recently identified as a glutathione-independent ferroptosis suppressor[34] and the superfamily of enzymes AKR, specially AKR1C1, AKR1C2 & AKR1C3.[13]

FSP1 acts as an oxidoreductase that reduces coenzyme Q10 to ubiquinol using NAD(P)H, thereby preventing lipid peroxidation in cell membranes[35]. It contains a flavin adenine dinucleotide (FAD)-binding domain and a NAD(P)H-binding domain, like other NADH oxidoreductase. A unique carboxy-terminal domain is crucial for its catalytic activity and ferroptosis inhibition by mediating dimerization and forming two active sites responsible for ubiquinone reduction and FAD hydroxylation. FSP1 catalyzes the production of hydrogen peroxide and converts FAD to 6-hydroxy-FAD in the presence of oxygen and NAD(P)H. 6-hydroxy-FAD acts as a potent radical-trapping antioxidant, directly inhibiting ferroptosis in cells[34].

In this context aldo-keto reductase enzymes (AKRs) play a crucial role in ferroptosis resistance, thus, human AKR is a superfamily of detoxifying enzymes that catalyze the conversion of aldehydes and ketones into their low-reactive primary and second alcohols, by using NAD(P)H as a cofactor[36]. AKR family display a catalytic activity in cellular signaling involved in the regulation of endogenous substrates such as lipid aldehydes, that explain its implicated in pathogenesis and neurodegenerative disease[37].

Downstream upregulation/activation of AKR1C1÷3C is a mechanism involving the inhibition of ferroptotic cell death through the reduction of lipid peroxide levels. Several studies indicated that AKRs are highly expressed in a series of tumors. Therefore, a new potential therapeutic strategy for effectively killing cancer cells could be based on pro-ferroptosis drugs coupled with AKR1C1 ÷3 inhibitors[13].

1.1.5 Ferroptosis and disease

Under physiological conditions, iron and ROS are important for cell proliferation and signal transduction. Under pathological conditions, and after exceeding a certain limit of damage, iron overload and oxidative damage can cause ferroptotic cell death[38].

Ferroptosis plays an important role in regulation of oxidative stress and inflammatory responses. Thus, ferroptosis is capable of releasing pro-inflammatory damage-associated molecular patterns (DAMPs) that promote sterile inflammation and the development of numerous inflammatory disease[39].

Acute ferroptosis participates in pathological cell loss as well as in malignant processes[38]. Recently, novel evidence highlights ferroptosis as a pivotal player in the development and progression of numerous organ injuries and disease such as cancer, ischemic organ injuries,[40] liver disease [41] and neurodegenerative pathologies, such as Parkinson's disease (PD)[42] Huntington's disease (HD), Alzheimer's disease (AD)[43], Multiple sclerosis (MS)[44,45] , cardiovascular diseases[38].

Since ferroptosis functions as tumour-suppression mechanism, dysregulated ferroptosis has been shown to be involved in cancer. Ferroptosis has been linked to cancer since the very beginning of this field of research: the initial discovery of chemical inducers of ferroptosis is the result of hunting for novel anti-cancer therapeutic compounds. Subsequent mechanistic studies have revealed that numerous cancer-relevant genes and signalling pathways regulate ferroptosis. Interestingly it has been observed that mesenchymal and dedifferentiated cancer cells, which are often resistant to apoptosis and common therapeutics, are highly susceptible to ferroptosis inducers. Conceptually, as ferroptosis is an oxidative-stress-induced form of cell death that is tightly interwoven with cell metabolism, it seems logical to propose that cancer cells may have higher tendency to undergo ferroptosis, due to overall more active metabolism and higher ROS load[45]. Additionally, it has been shown that cancer cells often demand high iron supply, which may further sensitize them to ferroptosis[46].

Nowadays, research focused on development of ferroptosis induction-based cancer therapies. To this aim, several untargeted nanoparticle-based strategies to deliver iron, peroxides, and other toxic cargoes to kill tumour cells have been tested. Since ferroptosis process is controlled by multiple enzymes, the development of targeted approaches is possible. Despite the most obvious target is recognized in GPX4, the low specificity of its inhibitors and the fact that selenoproteins are essential for various peripheral tissues, such as kidney tubular cells and neuronal subpopulation, targeting GPX4 will probably cause substantial side effects. Unlike targeting GPX4, approaches to limit cellular cyst(e)ine availability by inhibiting system Xc- are highly promising[45].

In addition, recent results identify ferroptosis as a detrimental and targetable factor in multiple sclerosis (MS). These findings create novel potential treatment options for MS patients. Characterized by progressive loss of neurons and oligodendrocytes[47].

1.2 Oligodendrocyte

In the human central nervous system (CNS), glial cells, also known as neuroglia, play divers and dynamic roles in orchestrating nearly all aspects of nervous system formation and functions. Glial cells include Radial glia, astrocytes, oligodendrocyte progenitor cells, oligodendrocytes and microglia[48].

Glial cells serve essential housekeeping functions, responding to stress conditions to protect CNS and preserve its normal function[49].

Among them, oligodendrocyte progenitor cells (OPCs) are the most proliferative cells in the CNS, continuously generating myelinating oligodendrocytes throughout life[48].

OPCs originate from the ventricular zone during early development, proliferate and migrate into the different developing areas of the brain, where they differentiate into myelin-forming Oligodendrocyte (Ols). Unlike most progenitor cells, OPCs persist throughout life as adult, self-renewing OPCs that can differentiate into newly formed myelinating Ols to maintain myelin plasticity or in response to damaging signals[50].

The OL differentiation passes through four stages: proliferative OPCs, pre-Ols, differentiated Ols and myelinating Ols. This process is controlled by the combination of OL-specific transcription factors, extracellular signals, epigenetic modification and specific signalling pathways, which homeostatic balance maintains proper differentiation[50].

In general, we can distinguish between positive regulators such as transcription factor (SOX10, OLID2 and ASCL1) which stimulate differentiation and support formation of myelin in central nervous system , and negative regulator (HES1, HES5 and ID2, ID4, SOX5 and SOX6) which act as an inhibitor of myelinating gene and maintain oligodendrocyte precursor cells (OPCs) in an undifferentiated state[50].

The differentiated OLs can be divided into two stages: pre-myelinating and myelinated Ols. The inhibition of some proteins and stimulation of others allow the formation of

myelin sheath around axons which is very essential for the proper function of CNS[51] (figure 3).

Current data indicate that the epigenetic mechanisms such as DNA methylation, histone modification, micro-RNAs (miRNAs) **-** including miR-125a, miR-23, miR-138, miR-338 and miR-219 - play an essential role in regulating the differentiation of OLs. These epigenetic signatures translate extracellular signals into functional cellular changes, also coordinating the transcriptional machinery responsible for the differentiation process[50,52].

Oligodendrocytes play a vital role in maintaining the integrity of myelin, and its proper function in efficient signal transmission, as well as, in preserving the structural and functional integrity of axons which is vital for overall neuronal health. Thus, OLs produce myelin, a fatty substance that forms a sheath around neuronal axons. Myelin sheath is crucial for enhancing the speed and efficiency of electrical signal transmission along neurons, also protecting axons from damage[53].

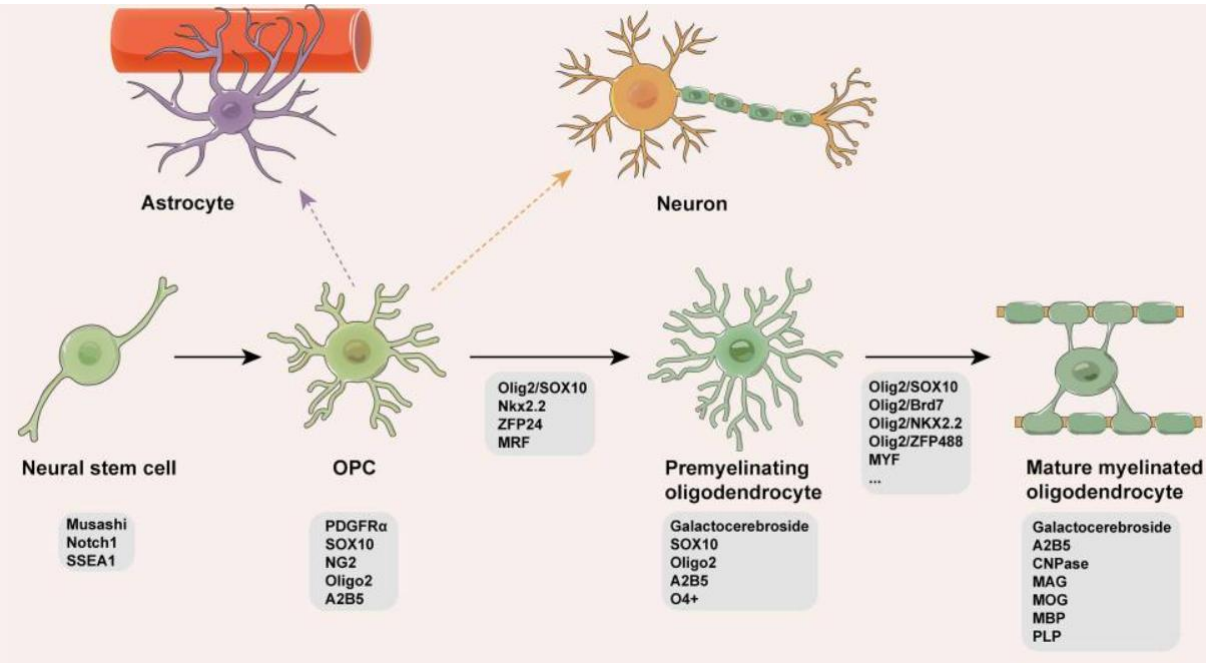

Figure 3: The origin and development of oligodendrocyte lineage cells start from neural stem cells, can be subdivided into three stages: oligodendrocyte progenitor cells (OPCs), pre-myelinating oligodendrocytes and mature oligodendrocytes (Ols).[51]

2. Project aim

The aims of the project are to:

1. evaluate the susceptibility of human mature OLs to ferroptosis induction and execution;
2. unveil the molecular mechanism(s) regulating the ferroptotic process in OLs.

All the experiments were carried out by using the human oligodendrocyte precursor cell line MO3.13 as a model

3. Result and discussion

1.3 Differentiation of MO3.13 cells

The difficulties of working with oligodendrocyte progenitor cells (OPCs), such as their intrinsic heterogeneity, variable differentiation trajectories into myelin oligodendrocytes (myOLs), and high cost, make MO3.13 cell lines an acceptable alternative to study the biology of OLs and their role in neurodegenerative disease at the molecular level. However, it is important to note that these cells fail to achieve terminal differentiation typical of myOLs.

In order to induce the phenotypic and metabolic characteristics of mature OLs, MO3.13 cells were differentiated, exposing cells to 100nM PMA, in a serum-free medium, for 3 days.

Undifferentiated (UND) or immature MO3.13 cells appear flat and less branched. While Mature (MAT) cells become more elongated and show branched structures (Fig 4, A).

Cell maturation was monitored at biochemical levels, by measuring the expression of well-known mature OLs markers such as OLIG1, BMP, NOX3, NOX5 and OLIG2[54,55]. The expression of these markers increased in mature MO3.13 (fig 4, B-C), in parallel with morphological changes (fig 4, A).

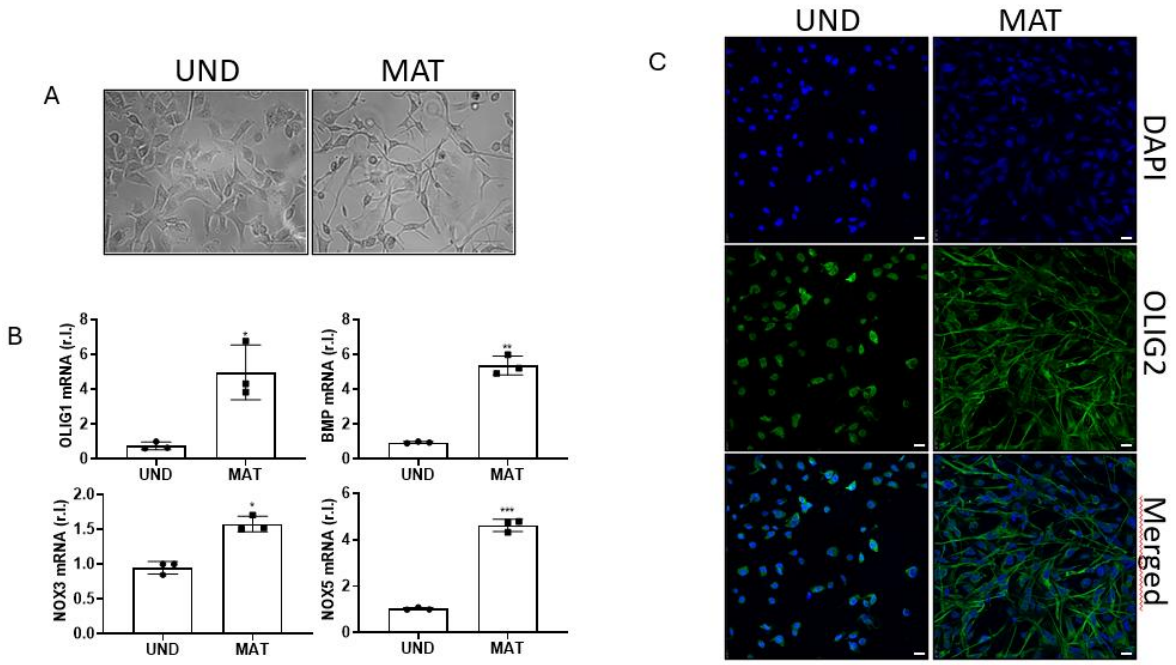

Figure 4: Validation of OLs differentiation. (A) Representative images of undifferentiated (on the right) and differentiated (on the left) MO3.13 cells. **(B)** mRNA levels of OLIG1, BMP, NOX3 and NOX5 were evaluated by qRT-PCR, in undifferentiated and differentiated MO3.13. L34 was used as internal control. **(C)** Representative images of immunofluorescence staining for oligodendrocyte maturation markers OLIG2 (green), while DAPI was used to stain nuclei, in MO3.13 cells. Experiments were performed in triplicate and repeated three times. Histograms represent the mean ± SD; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

2.3 Maturation of MO3.13 correlate with resistance to ferroptotic execution

Next, we evaluated the susceptibility of precursor MO3.13 to ferroptosis execution. Different treatments were applied to MO3.13 cells, and cell viability was measured at 18 and 24 hours by Alamar Blue.

In order to induce ferroptosis, UND MO3.13 were exposed to ERA (Erastin) or RSL3, in absence or presence of the specific ferroptotic inhibitors Deferoxamine (DFO) and Ferrostatin-1 (Fer), to confirm the ferroptotic cell death modality.

Deferoxamine is an iron chelator that prevents iron overload complications, while Ferrostatin (Fer) is a lipid peroxide scavenger.

Thus, results shown in Figure 5A clearly demonstrate that UND cells are susceptible to ferroptosis independently from the specific stimulus, while both DFO or Fer completely prevented ERA- or RSL3-induced cell death, confirming the induction of ferroptosis.

To evaluate the sensitivity of OLs during maturation, both undifferentiated and mature MO3.13 cells were treated with the same ferroptosis inducers: ERA and RSL3.

Interestingly, while UND cells were sensitive to both stimuli, mature OLs remained resistant to ferroptosis, maintaining high viability, indicating that maturation protect OLs from ferroptosis (Fig:5-B).

Since lipid peroxides are considered a hallmark of ferroptosis, a significant increase in lipid-ROS was observed in UND cells exposed to ERA or RSL3, while no accumulation was observed in mature OLs (Fig:5-C). These results indicate that mature OLs are protected from ferroptosis execution.

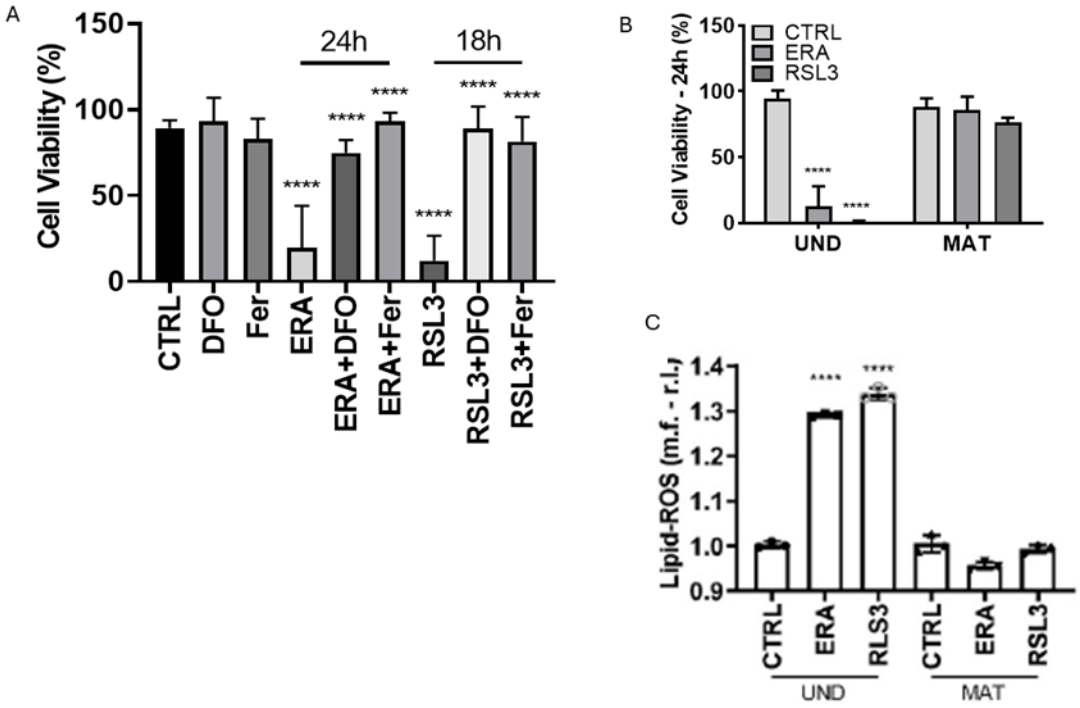

Figure 5: Evaluation of ferroptosis sensitivity in differentiated MO3.13 cells. **(A)** Undifferentiated M03.13 cell viability analysis after 24h from exposer to Erastin (ERA;1 µM) and after 18h from exposer to RSL3 (0.5 µM), both in presence or absence of Fer-1 (10 µM) or DFO (100 µM). **(B)** UND and MAT cells were exposed 24h to ERA (1 µM) or RSL3 (0.5 µM), and cell viability was evaluated by Alamar Blue. **(C)** UND and MAT MO3.13 were treated for 4h by ERA (1 µM) or RSL3 (0.5 µM) and lipids peroxides were evaluated by DOBIPY C11. Result show mean ± SD from three independent experiments; *P< 0.05; **P< 0.01; P< 0.001; ****P< 0.0001.

3.3 AKR1C1 expression increased in mature MO3.13 cells

Since mature OLs are resistant to ferroptosis, we aimed to investigate the underlying mechanism(s) responsible for this phenotype.

We previously identified members of the aldo-keto reductase (AKRs) superfamily of enzymes as ferroptosis inhibitors in metastatic melanoma[13].

AKRs are a family of enzymes capable of converting aldehydes and ketones into their corresponding less reactive alcohols. They are also involved in the reduction of lipid-ROS, thereby inhibiting ferroptosis. AKR1C1 is considered the main isoform of the superfamily of AKRs[56] enzymes expressed in the CNS[57].

To investigate whether AKRs are induced upon maturation of MO3.13, the expression of AKR1C1 was measured in undifferentiated and mature MO3.13 at both protein (Fig:6-A and B) and mRNA level (Fig:6-C). Data reported in Fig:6A-C show a clear increase in AKR1C1 expression in mature OLs compare to undifferentiated cells.

The maturation-dependent increase of AKR1C1 was further confirmed by measuring its enzymatic activity, using the Coumberol Assay. AKR1C1 catalyzes the conversion of Coumberone into Coumberol, and its activity can be monitored by measuring Coumberol levels. MPA, an inhibitor of AKR1C1 (10μM and 30μM), was used as a control. Figure 6-D show low levels of Coumberol in undifferentiated cells which confirm the low enzymatic activity of AKR1C1, in contrast, matured cells show increased Coumberol levels, indicating higher AKR1C1 activity. The conversion was inhibited by MPA, in a dose-dependent manner. These results support that AKR1C1 is highly expressed and enzymatically active in mature MO3.13 cells.

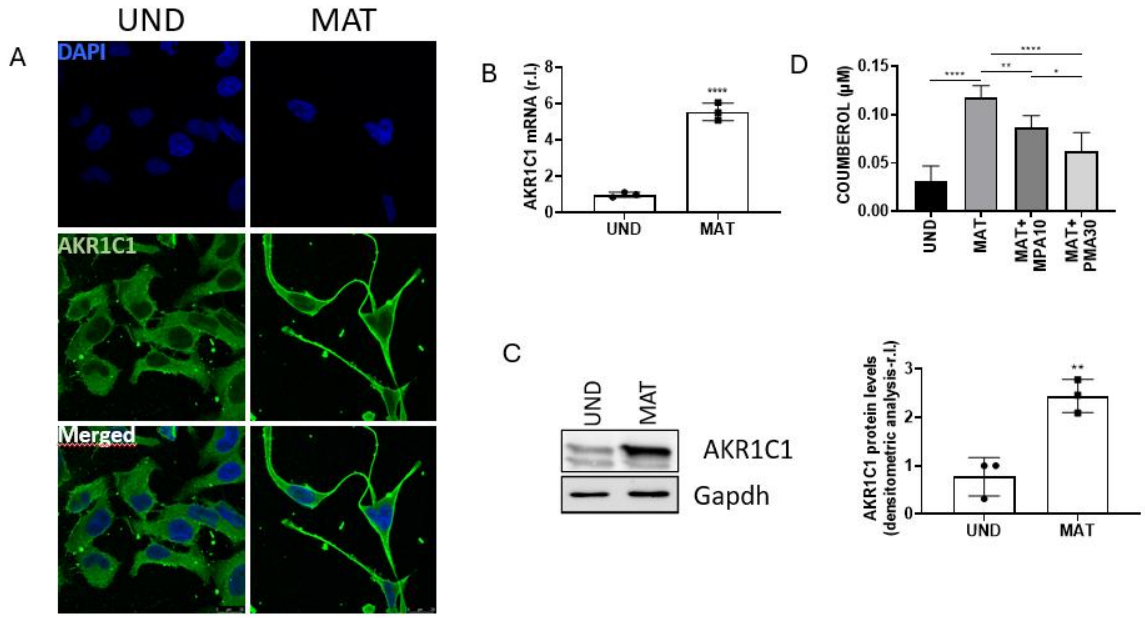

Figure 6: AKR1C1 is upregulated in mature Oligodendrocytes.

(A) Representative images of immunofluorescence staining for AKR1C1 (green) and DAPI (blue) of undifferentiated and mature MO3.13. **(B)** mRNA level of AKR1C1 was evaluated by qPCR analysis. **(C)** AKR1C1 protein levels were evaluated by western blot analysis. (D)AKRs enzymatic activity was measured evaluating the conversion of Coumberol in absence or presence of MPA (10 or 30 µM). Data represent mean ± SD; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

4.3 AKR1C1 confers resistance to ferroptosis execution of mature OLs

To confirm the role of AKR1C1 in the resistance of ferroptosis execution in mature OLs, cells were treated with the AKR1C1 specific inhibitor 3-bromo-5-phenyl salicylic acid (AS), and cell viability was evaluated at 24h post treatment. Data reported at Fig:7A show decrease cell viability with increasing concentrations of AS, supporting the role of AKR1C1 in protect the cell from ferroptosis.

Combined treatment including AKR1C1 inhibitor plus ferroptosis inducer guaranteed decrease cell viability. Cells treated with AS50+ ERA show significant decrease in cell viability Fig:7B.

Collectively, these data support the conclusion that AKR1C1 is a key mediator of ferroptosis resistance in mature OLs.

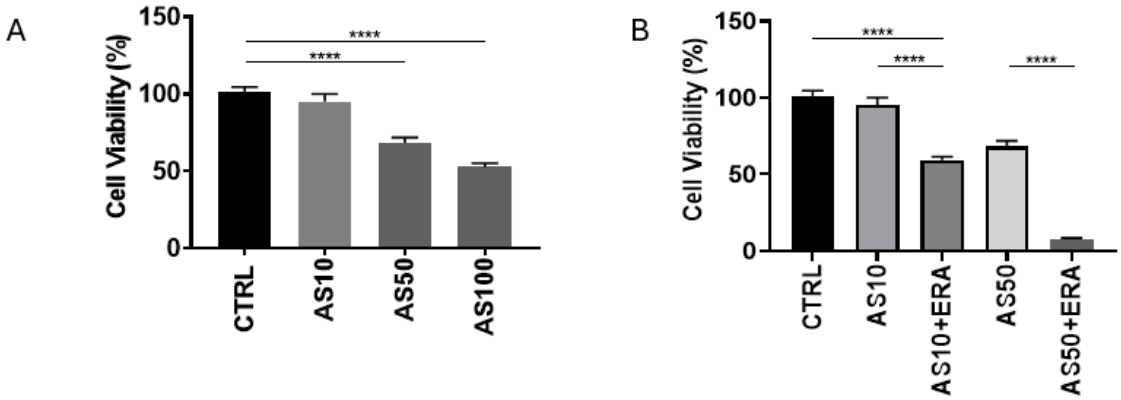

Figure 7: Functional role of AKR1C1 in ferroptosis resistance. (A) Mature OLs were treated 24h with AS (10, 50, 100 µM) and the cell viability was evaluated. **(B)** Mature OLs were treated 24h with ERA (1 µM) in combination with3-bromo-5-phenyl salicylic acid (AS;10 or 50 µM) and the cell viability was evaluated. Histograms represent the mean ± SD; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

4. Conclusion

Multiple sclerosis (MS) is considered the most common cause of non-traumatic neurological injury affecting quality of life, physical functioning, and cognitive

performance. Worldwide a total of 2.8 million people (35.9 per 100,000 population) are estimated to be affected by MS[47].

MS is a chronic autoimmune disease characterized by demyelination, inflammation, neuronal loss and gliosis[58] in the brain and spinal cord[59]. Demyelination with consequent axonal loss is the primary cause of MS progression. Thus, signals transmitted across nerves are slowed or blocked, causing neurological symptoms that can result in decreased quality of life and disability[59]. Taking in consideration that Oligodendrocytes play essential role in nerve conduction and overall neuronal health due to their function in producing myelin, finding new therapeutic avenues that prevent their death becomes a priority. [53]

The main goal of current MS treatments is to manage sudden symptoms, use medicines that slow down the disease. This shows that more research is needed to find better treatments. Right now, there aren't any treatments that focus on stopping cell death[58'47].

Some patients with progressive MS can keep their disease stable with treatment, they don't get worse, and there are no new symptoms or changes on MRI scans. This leads to an important question: is it possible to actually improve or reverse disability in these patients?

Recent studies show that the ferroptosis might be involved in the progressive neurodegeneration observed in Multiple Sclerosis.[47].

Our results shown that the maturation of oligodendrocytes confers a significant resistance to ferroptosis, a regulated form of iron-dependent cell death associated with oxidative stress. The findings demonstrate that mature OLs exhibit increased expression of AKR1C1 (aldo-keto reductase family 1 member C1), a key enzyme that confers protection against ferroptosis due to its role in detoxifying Lipid-ROS, the mai executioners of the ferroptotic process. The upregulation of AKR1C1 appears to be a key mechanism by which mature OLs evade ferroptotic cell death. These findings have important implications for understanding neurodegenerative and demyelinating disease, such as multiple sclerosis (MS), where oligodendrocyte death contributes to disease

progression. The protective role of AKR1C1 suggests that enhancing its expression or activity could be a potential therapeutic strategy to promote OL survival and remyelination in such disorders. Eventually, provide a new insight into therapeutic strategy that target ferroptosis execution for neurodegenerative disease and MS. Further research could explore whether AKR1C1 overexpression in animal models of demyelination improves remyelination and disease outcomes. Also examine AKR1C1 expression in human brain tissue from MS patients to validate its relevance in human disease.

5. Method

Cell culture and treatments

For these experiments were used MO3.13 cell lines and were cultured in Dulbecco's modified Eagle's medium (DMEM, EuroClone), supplemented with 10% Fetal Bovine Serum (FBS; Merck), 100U/ml penicillin, 100µg/ml streptomycin (Euroclone).

Cells were incubated at 37°C in 5% CO^2 atmosphere. MO3.13 cells were differentiated using serum-free DMEM supplemented with 100 nM phorbol 12-Myristate 13-Acetate (PMA; Merck) for 3 days. Cells were treated with erastin 1µM (ERA; Merck), RSL3 0,5 µM (Merck), Deferoxamine 100µM (DFO; Cayman Chemicals), Ferrostatin-1 10µM (Fer-1; Merck), 3- bromo-5-phenyl salicylic acid 10–50 µM (AS; Merck).

All agents were diluted in DMSO or PBS. Control cells were treated with the corresponding vehicle in equal volum.

Quantitative PCR (qPCR)

Total RNA was isolated using TripleXtactor reagent (Smobio) following the manufacturer's recommendations. cDNA synthesis was performed using the AMV Reverse Transcriptase Kit (Promega) by using 2µg of total RNA. Rt-qPCR was conducted on a CFX96 thermocycler (Bio-Rad) to produce fluorescently labelled PCR products during repetitive cycling of the amplification reaction using Maxima SYBR Green q PCR Master Mix (Smobio), a melting curve was used to verify amplification specificity. Primers were designed using IDT PrimerQuest Tool. Primers sequences were designed by using the online IDT pri-merQuest Tool software, and sequences reported below:

Gene	Forward primer	Reverse primer
NOX5	GGGTGACTCAGCAGTTTAAG	GTGATGGTGCCACTTCTATC
NOX3	GAGGGTCTCTCCACCATATT	TTGAGGTAGCTCTCGTTAGG
OLIG1	CCCAGCAGTAGGATGTAGT	GTCATCCTGCCCTACTCA
MBP	CTGTCCCTGAGCAGATTTAG	CCCTTGTGAGCCGATTTAT
AKR1C1	GCCGTGGAGAAGTGTAAAG	CAGACAGGCTTGTACTTGAG
L34	GTCCCGAACCCCTGGTAATAGA	GGCCCTGCTGACATGTTTCTT

Results were expressed as the treshold cycle (CT). The ΔCT is the difference between the CT for the specific mRNA and the CT for the reference mRNA, L34. To determine relative mRNA levels, 2 was raised to the power of ΔΔCT (the difference between the ΔCT from treated cells and the ΔCT from untreated cells).

Wastern blotting

Proteins were isolated by using a RIPA buffer (50mM Tris-HCL, ph7.5, 150mM NaCl,1% NP40, 0.5%deoxycholate, 0.1% SDS) supplemented with a protease inhibitor cocktail (Sigma), and an equal amount of proteins (30μg) were subjected to an SDS-PAGE, and electroblotted onto nitrocellulose membranes (Bio-Rad). Membranes were blocked 2h by using 5% non -fat dry milk (Merck) in PBS plus 0.1% Tween20 (Merck) and incubated with the indicated primary antibodies in blocking solution, overnight at 4 C: anti-Olig2(1:500, Millipore), anti-GAPDH (1:500, Santa Cruz Biotechnology),), anti-AKR1C1 (1:500; OriGene). Detection was achieved using horseradish peroxidase-conjugate secondary antibody (1:5000; Jackson ImmunoResearch) and visualized with ECL plus

(Amersham Biosciences). Images were acquired by using a ChemiDocTM Touch Imaging System (Bio-Rad) and analyzed by Image Lab software (Bio-Rad).

Immunofluorescence

Cells were grown on glass coverslips, coated with1% fibronectin (Merck) and 1% collagen (Merck), under experimental conditions, as described. Olig-2 detection: cells were fixed in 4% PFA (Merck) in PBS, supplemented with 2% sucrose (Merck), PH 7.4, for 15 min at room temperature. Samples were washes in PBS plus 2% sucrose and permeabilized for 10 min at 4°C with 20mM Hepes (Merck), 300mM Sucrose (Merck), 50mM NaCl (Merck), 3mM MgCl2 (Merck), plus 0.1% Triton X-100 (Merck). Cells were then incubated 30 min at 4°C with blocking buffer consisting in 20% FBS in PBS. Samples were incubated with primary rabbit polyclonal anti human Olig-2 antibody (Millipore), 1h (rt), washed and labelled with secondary 488 anti-rabbit IgG, 300 min, rt.

AKR1C1 staining samples were washed with cold PBS and fixed in PFA 4% for 15 min at 4°C, permeabilized with 0.5% Triton X-100 in cold PBS for 10 min at 4°C, washed twice and incubated with 10% donkey serum (Jackson ImmunoResearch) plus 0.05% Triton X-100 in cold PBS, 30 min at 4°C. Next, the slides were washed and incubated with anti-AKR primary antibody in 1% donkey serum plus 0.05% Triton X-100 in cold PBS, 1h at 4°C. After washing, the slides were incubated with appropriate secondary antibodies (Jackson ImmunoResearch) 1:500 in 1% donkey serum plus 0.05% Triton X-100 in cold PBS and incubated for 1h at 4°C. Coverslips were mounted onto glass using ProLong Gold Antifade with DAPI mounting solution (Thermo Fisher).

Images were acquired using a THUNDER 3D cell Imager (Leica) or SP8 confocal microscope (Leica).

Measurement of AKRS enzymatic activity

35 x 10^3 cells/well were seeded in 12-wellplate and supplemented in complete media (DEME w/o phenol red plus 100U/ml penicillin, 100µg/ml streptomycin, 4mM L-

glutamine, and 10% FBS) containing coumberone (10µM; MedChemExpress) ± MPA (10 or 30µM; Cayman Chemicals). The fluorescence of the cell medium was recorded after 3h, by using a 96-wellplate, by a Cytation 5 plate reader (biotek). Metabolic conversion rate was obtained by detecting fluorescence at 510 nm upon excitation at 385 nm. Coumberol was quantified using 0.1-10 µM Coumberol calibration curves in culture medium.

Cell viability

Cell viability was measured using AlamarBlueTM reagent (Bio-Rad) according to manufacturer's instructions. Briefly, 35 x 10^3 cells/well were plated in 24-well plates, treated as indicated, cell medium was discarded and an appropriate amount of Alamarblue reagent was added. cells were incubated 4 h, and fluorescence was monitored (530-560 nm excitation, and 590 nm emission wavelength) using a TECAN automation platform.

Lipid peroxides evaluation

35 x 10^3 cells were treated as indicated and cells harvested at indicate time points. Then, cells were washed with PBS and stained with BODIPY C11 (2 µM in PBS; Invitrogen) 15 min at 37 ◦C in the dark. Cells were pelleted and resuspended in PBS and 10 x 10^3 events were acquired by using a FACSympony (Beckton Dickinson). Data analysis was performed using the following software.

Bibliography

(1) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; Morrison, B.; Stockwell, B. R. Ferroptosis: An Iron-Dependent Form of Non-Apoptotic Cell Death. *Cell* **2012**, *149* (5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042.

(2) Dolma, S.; Lessnick, S. L.; Hahn, W. C.; Stockwell, B. R. Identification of Genotype-Selective Antitumor Agents Using Synthetic Lethal Chemical Screening in Engineered Human Tumor Cells. *Cancer Cell* **2003**, *3* (3), 285–296. https://doi.org/10.1016/s1535-6108(03)00050-3.

(3) Yang, W. S.; Stockwell, B. R. Synthetic Lethal Screening Identifies Compounds Activating Iron-Dependent, Nonapoptotic Cell Death in Oncogenic-RAS-Harboring Cancer Cells. *Chem. Biol.* **2008**, *15* (3), 234–245. https://doi.org/10.1016/j.chembiol.2008.02.010.

(4) Kroemer, G.; Galluzzi, L.; Vandenabeele, P.; Abrams, J.; Alnemri, E. S.; Baehrecke, E. H.; Blagosklonny, M. V.; El-Deiry, W. S.; Golstein, P.; Green, D. R.; Hengartner, M.; Knight, R. A.; Kumar, S.; Lipton, S. A.; Malorni, W.; Nuñez, G.; Peter, M. E.; Tschopp, J.; Yuan, J.; Piacentini, M.; Zhivotovsky, B.; Melino, G.; Nomenclature Committee on Cell Death 2009. Classification of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* **2009**, *16* (1), 3–11. https://doi.org/10.1038/cdd.2008.150.

(5) Galluzzi, L.; Vitale, I.; Aaronson, S. A.; Abrams, J. M.; Adam, D.; Agostinis, P.; Alnemri, E. S.; Altucci, L.; Amelio, I.; Andrews, D. W.; Annicchiarico-Petruzzelli, M.; Antonov, A. V.; Arama, E.; Baehrecke, E. H.; Barlev, N. A.; Bazan, N. G.; Bernassola, F.; Bertrand, M. J. M.; Bianchi, K.; Blagosklonny, M. V.; Blomgren, K.; Borner, C.; Boya, P.; Brenner, C.; Campanella, M.; Candi, E.; Carmona-Gutierrez, D.; Cecconi, F.; Chan, F. K.-M.; Chandel, N. S.; Cheng, E. H.; Chipuk, J. E.; Cidlowski, J. A.; Ciechanover, A.; Cohen, G. M.; Conrad, M.; Cubillos-Ruiz, J. R.; Czabotar, P. E.; D'Angiolella, V.; Dawson, T. M.; Dawson, V. L.; De Laurenzi, V.; De Maria, R.; Debatin, K.-M.; DeBerardinis, R. J.; Deshmukh, M.; Di Daniele, N.; Di Virgilio, F.; Dixit, V. M.; Dixon, S. J.; Duckett, C. S.; Dynlacht, B. D.; El-Deiry, W. S.; Elrod, J. W.; Fimia, G. M.; Fulda, S.; García-Sáez, A. J.; Garg, A. D.; Garrido, C.; Gavathiotis, E.; Golstein, P.; Gottlieb, E.; Green, D. R.; Greene, L. A.; Gronemeyer, H.; Gross, A.; Hajnoczky, G.; Hardwick, J. M.; Harris, I. S.; Hengartner, M. O.; Hetz, C.; Ichijo, H.; Jäättelä, M.; Joseph, B.; Jost, P. J.; Juin, P. P.; Kaiser, W. J.; Karin, M.; Kaufmann, T.; Kepp, O.; Kimchi, A.; Kitsis, R. N.; Klionsky, D. J.; Knight, R. A.; Kumar, S.; Lee, S. W.; Lemasters, J. J.; Levine, B.; Linkermann, A.; Lipton, S. A.; Lockshin, R. A.; López-Otín, C.; Lowe, S. W.; Luedde, T.; Lugli, E.; MacFarlane, M.; Madeo, F.; Malewicz, M.; Malorni, W.; Manic, G.; Marine, J.-C.; Martin, S. J.; Martinou, J.-C.; Medema, J. P.; Mehlen, P.; Meier, P.; Melino, S.; Miao, E. A.; Molkentin, J. D.; Moll, U. M.; Muñoz-Pinedo, C.; Nagata, S.; Nuñez, G.; Oberst, A.; Oren, M.; Overholtzer, M.; Pagano, M.; Panaretakis, T.; Pasparakis, M.; Penninger, J. M.; Pereira, D. M.; Pervaiz, S.; Peter, M. E.; Piacentini, M.; Pinton, P.; Prehn, J. H. M.; Puthalakath, H.; Rabinovich, G. A.; Rehm, M.; Rizzuto, R.; Rodrigues, C. M. P.; Rubinsztein, D. C.; Rudel, T.; Ryan, K. M.; Sayan, E.; Scorrano, L.; Shao, F.; Shi, Y.; Silke, J.; Simon, H.-U.; Sistigu, A.; Stockwell, B. R.; Strasser, A.; Szabadkai, G.; Tait, S. W. G.; Tang, D.; Tavernarakis, N.; Thorburn, A.; Tsujimoto, Y.; Turk, B.; Vanden Berghe, T.; Vandenabeele, P.; Vander Heiden, M. G.; Villunger, A.; Virgin, H. W.; Vousden, K. H.; Vucic, D.; Wagner, E. F.; Walczak, H.; Wallach, D.; Wang, Y.; Wells, J. A.; Wood, W.; Yuan, J.; Zakeri, Z.; Zhivotovsky, B.; Zitvogel, L.; Melino, G.; Kroemer, G. Molecular Mechanisms of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* **2018**, *25* (3), 486–541. https://doi.org/10.1038/s41418-017-0012-4.

(6) Kagan, V. E.; Mao, G.; Qu, F.; Angeli, J. P. F.; Doll, S.; Croix, C. S.; Dar, H. H.; Liu, B.; Tyurin, V. A.; Ritov, V. B.; Kapralov, A. A.; Amoscato, A. A.; Jiang, J.; Anthonymuthu, T.; Mohammadyani, D.; Yang, Q.; Proneth, B.; Klein-Seetharaman, J.; Watkins, S.; Bahar, I.; Greenberger, J.; Mallampalli, R. K.; Stockwell, B. R.; Tyurina, Y. Y.; Conrad, M.; Bayır, H. Oxidized Arachidonic and Adrenic PEs Navigate Cells to Ferroptosis. *Nat. Chem. Biol.* **2017**, *13* (1), 81–90. https://doi.org/10.1038/nchembio.2238.

(7) Ingold, I.; Berndt, C.; Schmitt, S.; Doll, S.; Poschmann, G.; Buday, K.; Roveri, A.; Peng, X.; Porto Freitas, F.; Seibt, T.; Mehr, L.; Aichler, M.; Walch, A.; Lamp, D.; Jastroch, M.; Miyamoto, S.; Wurst, W.; Ursini, F.; Arnér, E. S. J.; Fradejas-Villar, N.; Schweizer, U.; Zischka, H.; Friedmann Angeli, J. P.; Conrad, M. Selenium Utilization by GPX4 Is Required to Prevent Hydroperoxide-Induced Ferroptosis. *Cell* **2018**, *172* (3), 409-422.e21. https://doi.org/10.1016/j.cell.2017.11.048.

(8) Shah, R.; Shchepinov, M. S.; Pratt, D. A. Resolving the Role of Lipoxygenases in the Initiation and Execution of Ferroptosis. *ACS Cent. Sci.* **2018**, *4* (3), 387–396. https://doi.org/10.1021/acscentsci.7b00589.

(9) Ward, D. M.; Kaplan, J. Ferroportin-Mediated Iron Transport: Expression and Regulation. *Biochim. Biophys. Acta* **2012**, *1823* (9), 1426–1433. https://doi.org/10.1016/j.bbamcr.2012.03.004.

(10) Gao, M.; Monian, P.; Quadri, N.; Ramasamy, R.; Jiang, X. Glutaminolysis and Transferrin Regulate Ferroptosis. *Mol. Cell* **2015**, *59* (2), 298–308. https://doi.org/10.1016/j.molcel.2015.06.011.

(11) Mancias, J. D.; Wang, X.; Gygi, S. P.; Harper, J. W.; Kimmelman, A. C. Quantitative Proteomics Identifies NCOA4 as the Cargo Receptor Mediating Ferritinophagy. *Nature* **2014**, *509* (7498), 105–109. https://doi.org/10.1038/nature13148.

(12) Mancias, J. D.; Pontano Vaites, L.; Nissim, S.; Biancur, D. E.; Kim, A. J.; Wang, X.; Liu, Y.; Goessling, W.; Kimmelman, A. C.; Harper, J. W. Ferritinophagy via NCOA4 Is Required for Erythropoiesis and Is Regulated by Iron Dependent HERC2-Mediated Proteolysis. *eLife* **2015**, *4*, e10308. https://doi.org/10.7554/eLife.10308.

(13) Gagliardi, M.; Saverio, V.; Monzani, R.; Ferrari, E.; Piacentini, M.; Corazzari, M. Ferroptosis: A New Unexpected Chance to Treat Metastatic Melanoma? *Cell Cycle Georget. Tex* **2020**, *19* (19), 2411–2425. https://doi.org/10.1080/15384101.2020.1806426.

(14) Yang, W. S.; Kim, K. J.; Gaschler, M. M.; Patel, M.; Shchepinov, M. S.; Stockwell, B. R. Peroxidation of Polyunsaturated Fatty Acids by Lipoxygenases Drives Ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (34), E4966-4975. https://doi.org/10.1073/pnas.1603244113.

(15) Haeggström, J. Z.; Funk, C. D. Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. *Chem. Rev.* **2011**, *111* (10), 5866–5898. https://doi.org/10.1021/cr200246d.

(16) Yan, S.; Yang, X.-F.; Liu, H.-L.; Fu, N.; Ouyang, Y.; Qing, K. Long-Chain Acyl-CoA Synthetase in Fatty Acid Metabolism Involved in Liver and Other Diseases: An Update. *World J. Gastroenterol.* **2015**, *21* (12), 3492–3498. https://doi.org/10.3748/wjg.v21.i12.3492.

(17) Xie, Y.; Hou, W.; Song, X.; Yu, Y.; Huang, J.; Sun, X.; Kang, R.; Tang, D. Ferroptosis: Process and Function. *Cell Death Differ.* **2016**, *23* (3), 369–379. https://doi.org/10.1038/cdd.2015.158.

(18) Birben, E.; Sahiner, U. M.; Sackesen, C.; Erzurum, S.; Kalayci, O. Oxidative Stress and Antioxidant Defense. *World Allergy Organ. J.* **2012**, *5* (1), 9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

(19) Rochette, L.; Dogon, G.; Rigal, E.; Zeller, M.; Cottin, Y.; Vergely, C. Lipid Peroxidation and Iron Metabolism: Two Corner Stones in the Homeostasis Control of Ferroptosis. *Int. J. Mol. Sci.* **2022**, *24* (1), 449. https://doi.org/10.3390/ijms24010449.

(20) Sato, H.; Tamba, M.; Ishii, T.; Bannai, S. Cloning and Expression of a Plasma Membrane Cystine/Glutamate Exchange Transporter Composed of Two Distinct Proteins. *J. Biol. Chem.* **1999**, *274* (17), 11455–11458. https://doi.org/10.1074/jbc.274.17.11455.
(21) Jaramillo, M. C.; Zhang, D. D. The Emerging Role of the Nrf2-Keap1 Signaling Pathway in Cancer. *Genes Dev.* **2013**, *27* (20), 2179–2191. https://doi.org/10.1101/gad.225680.113.
(22) Jiang, L.; Kon, N.; Li, T.; Wang, S.-J.; Su, T.; Hibshoosh, H.; Baer, R.; Gu, W. Ferroptosis as a P53-Mediated Activity during Tumour Suppression. *Nature* **2015**, *520* (7545), 57–62. https://doi.org/10.1038/nature14344.
(23) Had-Aissouni, L. Toward a New Role for Plasma Membrane Sodium-Dependent Glutamate Transporters of Astrocytes: Maintenance of Antioxidant Defenses beyond Extracellular Glutamate Clearance. *Amino Acids* **2012**, *42* (1), 181–197. https://doi.org/10.1007/s00726-011-0863-9.
(24) Xu, T.; Ding, W.; Ji, X.; Ao, X.; Liu, Y.; Yu, W.; Wang, J. Molecular Mechanisms of Ferroptosis and Its Role in Cancer Therapy. *J. Cell. Mol. Med.* **2019**, *23* (8), 4900–4912. https://doi.org/10.1111/jcmm.14511.
(25) Yant, L. J.; Ran, Q.; Rao, L.; Van Remmen, H.; Shibatani, T.; Belter, J. G.; Motta, L.; Richardson, A.; Prolla, T. A. The Selenoprotein GPX4 Is Essential for Mouse Development and Protects from Radiation and Oxidative Damage Insults. *Free Radic. Biol. Med.* **2003**, *34* (4), 496–502. https://doi.org/10.1016/s0891-5849(02)01360-6.
(26) Brigelius-Flohé, R.; Maiorino, M. Glutathione Peroxidases. *Biochim. Biophys. Acta* **2013**, *1830* (5), 3289–3303. https://doi.org/10.1016/j.bbagen.2012.11.020.
(27) Sui, X.; Zhang, R.; Liu, S.; Duan, T.; Zhai, L.; Zhang, M.; Han, X.; Xiang, Y.; Huang, X.; Lin, H.; Xie, T. RSL3 Drives Ferroptosis Through GPX4 Inactivation and ROS Production in Colorectal Cancer. *Front. Pharmacol.* **2018**, *9*, 1371. https://doi.org/10.3389/fphar.2018.01371.
(28) Yang, W. S.; SriRamaratnam, R.; Welsch, M. E.; Shimada, K.; Skouta, R.; Viswanathan, V. S.; Cheah, J. H.; Clemons, P. A.; Shamji, A. F.; Clish, C. B.; Brown, L. M.; Girotti, A. W.; Cornish, V. W.; Schreiber, S. L.; Stockwell, B. R. Regulation of Ferroptotic Cancer Cell Death by GPX4. *Cell* **2014**, *156* (1–2), 317–331. https://doi.org/10.1016/j.cell.2013.12.010.
(29) Kinowaki, Y.; Kurata, M.; Ishibashi, S.; Ikeda, M.; Tatsuzawa, A.; Yamamoto, M.; Miura, O.; Kitagawa, M.; Yamamoto, K. Glutathione Peroxidase 4 Overexpression Inhibits ROS-Induced Cell Death in Diffuse Large B-Cell Lymphoma. *Lab. Investig. J. Tech. Methods Pathol.* **2018**, *98* (5), 609–619. https://doi.org/10.1038/s41374-017-0008-1.
(30) Mou, Y.; Wang, J.; Wu, J.; He, D.; Zhang, C.; Duan, C.; Li, B. Ferroptosis, a New Form of Cell Death: Opportunities and Challenges in Cancer. *J. Hematol. Oncol.J Hematol Oncol* **2019**, *12* (1), 34. https://doi.org/10.1186/s13045-019-0720-y.
(31) Liu, J.; Kang, R.; Tang, D. Signaling Pathways and Defense Mechanisms of Ferroptosis. *FEBS J.* **2022**, *289* (22), 7038–7050. https://doi.org/10.1111/febs.16059.
(32) Nguyen, T.; Nioi, P.; Pickett, C. B. The Nrf2-Antioxidant Response Element Signaling Pathway and Its Activation by Oxidative Stress. *J. Biol. Chem.* **2009**, *284* (20), 13291–13295. https://doi.org/10.1074/jbc.R900010200.
(33) Ma, Q. Role of Nrf2 in Oxidative Stress and Toxicity. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320.
(34) Lv, Y.; Liang, C.; Sun, Q.; Zhu, J.; Xu, H.; Li, X.; Li, Y.-Y.; Wang, Q.; Yuan, H.; Chu, B.; Zhu, D. Structural Insights into FSP1 Catalysis and Ferroptosis Inhibition. *Nat. Commun.* **2023**, *14* (1), 5933. https://doi.org/10.1038/s41467-023-41626-7.
(35) Zhang, Y.; Hu, K.; Shang, Z.; Yang, X.; Cao, L. Ferroptosis: Regulatory Mechanisms and Potential Targets for Bone Metabolism: A Review. *Medicine (Baltimore)* **2024**, *103* (39), e39158. https://doi.org/10.1097/MD.0000000000039158.
(36) Pollak, N.; Dölle, C.; Ziegler, M. The Power to Reduce: Pyridine Nucleotides--Small Molecules with a Multitude of Functions. *Biochem. J.* **2007**, *402* (2), 205–218. https://doi.org/10.1042/BJ20061638.

(37) Penning, T. M.; Drury, J. E. Human Aldo-Keto Reductases: Function, Gene Regulation, and Single Nucleotide Polymorphisms. *Arch. Biochem. Biophys.* **2007**, *464* (2), 241–250. https://doi.org/10.1016/j.abb.2007.04.024.
(38) Tang, D.; Chen, X.; Kang, R.; Kroemer, G. Ferroptosis: Molecular Mechanisms and Health Implications. *Cell Res.* **2021**, *31* (2), 107–125. https://doi.org/10.1038/s41422-020-00441-1.
(39) Yu, Y.; Yan, Y.; Niu, F.; Wang, Y.; Chen, X.; Su, G.; Liu, Y.; Zhao, X.; Qian, L.; Liu, P.; Xiong, Y. Ferroptosis: A Cell Death Connecting Oxidative Stress, Inflammation and Cardiovascular Diseases. *Cell Death Discov.* **2021**, *7* (1), 193. https://doi.org/10.1038/s41420-021-00579-w.
(40) Stockwell, B. R.; Jiang, X.; Gu, W. Emerging Mechanisms and Disease Relevance of Ferroptosis. *Trends Cell Biol.* **2020**, *30* (6), 478–490. https://doi.org/10.1016/j.tcb.2020.02.009.
(41) Chen, J.; Li, X.; Ge, C.; Min, J.; Wang, F. The Multifaceted Role of Ferroptosis in Liver Disease. *Cell Death Differ.* **2022**, *29* (3), 467–480. https://doi.org/10.1038/s41418-022-00941-0.
(42) Ding, X.-S.; Gao, L.; Han, Z.; Eleuteri, S.; Shi, W.; Shen, Y.; Song, Z.-Y.; Su, M.; Yang, Q.; Qu, Y.; Simon, D. K.; Wang, X.-L.; Wang, B. Ferroptosis in Parkinson's Disease: Molecular Mechanisms and Therapeutic Potential. *Ageing Res. Rev.* **2023**, *91*, 102077. https://doi.org/10.1016/j.arr.2023.102077.
(43) Stockwell, B. R. Ferroptosis Turns 10: Emerging Mechanisms, Physiological Functions, and Therapeutic Applications. *Cell* **2022**, *185* (14), 2401–2421. https://doi.org/10.1016/j.cell.2022.06.003.
(44) Luoqian, J.; Yang, W.; Ding, X.; Tuo, Q.-Z.; Xiang, Z.; Zheng, Z.; Guo, Y.-J.; Li, L.; Guan, P.; Ayton, S.; Dong, B.; Zhang, H.; Hu, H.; Lei, P. Ferroptosis Promotes T-Cell Activation-Induced Neurodegeneration in Multiple Sclerosis. *Cell. Mol. Immunol.* **2022**, *19* (8), 913–924. https://doi.org/10.1038/s41423-022-00883-0.
(45) Jiang, X.; Stockwell, B. R.; Conrad, M. Ferroptosis: Mechanisms, Biology and Role in Disease. *Nat. Rev. Mol. Cell Biol.* **2021**, *22* (4), 266–282. https://doi.org/10.1038/s41580-020-00324-8.
(46) Manz, D. H.; Blanchette, N. L.; Paul, B. T.; Torti, F. M.; Torti, S. V. Iron and Cancer: Recent Insights. *Ann. N. Y. Acad. Sci.* **2016**, *1368* (1), 149–161. https://doi.org/10.1111/nyas.13008.
(47) Van San, E.; Debruyne, A. C.; Veeckmans, G.; Tyurina, Y. Y.; Tyurin, V. A.; Zheng, H.; Choi, S. M.; Augustyns, K.; van Loo, G.; Michalke, B.; Venkataramani, V.; Toyokuni, S.; Bayir, H.; Vandenabeele, P.; Hassannia, B.; Vanden Berghe, T. Ferroptosis Contributes to Multiple Sclerosis and Its Pharmacological Targeting Suppresses Experimental Disease Progression. *Cell Death Differ.* **2023**, *30* (9), 2092–2103. https://doi.org/10.1038/s41418-023-01195-0.
(48) Allen, N. J.; Lyons, D. A. Glia as Architects of Central Nervous System Formation and Function. *Science* **2018**, *362* (6411), 181–185. https://doi.org/10.1126/science.aat0473.
(49) Pistono, C.; Bister, N.; Stanová, I.; Malm, T. Glia-Derived Extracellular Vesicles: Role in Central Nervous System Communication in Health and Disease. *Front. Cell Dev. Biol.* **2020**, *8*, 623771. https://doi.org/10.3389/fcell.2020.623771.
(50) Tiane, A.; Schepers, M.; Rombaut, B.; Hupperts, R.; Prickaerts, J.; Hellings, N.; van den Hove, D.; Vanmierlo, T. From OPC to Oligodendrocyte: An Epigenetic Journey. *Cells* **2019**, *8* (10), 1236. https://doi.org/10.3390/cells8101236.
(51) Zhou, B.; Zhu, Z.; Ransom, B. R.; Tong, X. Oligodendrocyte Lineage Cells and Depression. *Mol. Psychiatry* **2021**, *26* (1), 103–117. https://doi.org/10.1038/s41380-020-00930-0.
(52) Elbaz, B.; Popko, B. Molecular Control of Oligodendrocyte Development. *Trends Neurosci.* **2019**, *42* (4), 263–277. https://doi.org/10.1016/j.tins.2019.01.002.
(53) López-Muguruza, E.; Matute, C. Alterations of Oligodendrocyte and Myelin Energy Metabolism in Multiple Sclerosis. *Int. J. Mol. Sci.* **2023**, *24* (16), 12912. https://doi.org/10.3390/ijms241612912.

(54) Cavaliere, F.; Urra, O.; Alberdi, E.; Matute, C. Oligodendrocyte Differentiation from Adult Multipotent Stem Cells Is Modulated by Glutamate. *Cell Death Dis.* **2012**, *3* (2), e268. https://doi.org/10.1038/cddis.2011.144.
(55) Accetta, R.; Damiano, S.; Morano, A.; Mondola, P.; Paternò, R.; Avvedimento, E. V.; Santillo, M. Reactive Oxygen Species Derived from NOX3 and NOX5 Drive Differentiation of Human Oligodendrocytes. *Front. Cell. Neurosci.* **2016**, *10*, 146. https://doi.org/10.3389/fncel.2016.00146.
(56) Gagliardi, M.; Cotella, D.; Santoro, C.; Corà, D.; Barlev, N. A.; Piacentini, M.; Corazzari, M. Aldo-Keto Reductases Protect Metastatic Melanoma from ER Stress-Independent Ferroptosis. *Cell Death Dis.* **2019**, *10* (12), 902. https://doi.org/10.1038/s41419-019-2143-7.
(57) Noorbakhsh, F.; Ellestad, K. K.; Maingat, F.; Warren, K. G.; Han, M. H.; Steinman, L.; Baker, G. B.; Power, C. Impaired Neurosteroid Synthesis in Multiple Sclerosis. *Brain J. Neurol.* **2011**, *134* (Pt 9), 2703–2721. https://doi.org/10.1093/brain/awr200.
(58) Haki, M.; AL-Biati, H. A.; Al-Tameemi, Z. S.; Ali, I. S.; Al-hussaniy, H. A. Review of Multiple Sclerosis: Epidemiology, Etiology, Pathophysiology, and Treatment. *Medicine (Baltimore)* **2024**, *103* (8), e37297. https://doi.org/10.1097/MD.0000000000037297.
(59) Marcus, R. What Is Multiple Sclerosis? *JAMA* **2022**, *328* (20), 2078. https://doi.org/10.1001/jama.2022.14236.