

OPINION

Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion

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Abstract | Ferroptosis is a recently recognized cell death modality that is morphologically, biochemically and genetically distinct from other forms of cell death and that has emerged to play an important role in cancer biology. Recent discoveries have highlighted the metabolic plasticity of cancer cells and have provided intriguing insights into how metabolic rewiring is a critical event for the persistence, dedifferentiation and expansion of cancer cells. In some cases, this metabolic reprogramming has been linked to an acquired sensitivity to ferroptosis, thus opening up new opportunities to treat therapy-insensitive tumours. However, it is not yet clear what metabolic determinants are critical for therapeutic resistance and evasion of immune surveillance. Therefore, a better understanding of the processes that regulate ferroptosis sensitivity should ultimately aid in the discovery of novel therapeutic strategies to improve cancer treatment. In this Perspectives article, we provide an overview of the known mechanisms that regulate sensitivity to ferroptosis in cancer cells and how the modulation of metabolic pathways controlling ferroptosis might reshape the tumour niche, leading to an immunosuppressive microenvironment that promotes tumour growth and progression.

Ferroptosis is a form of necrotic cell death marked by the oxidative modification of phospholipid membranes via an iron-dependent mechanism¹. An initial characterization of this pathway demonstrated that cysteine depletion, which leads to the exhaustion of the intracellular pool of glutathione (reduced) (GSH) specifically triggers this form of cell death². The requirement for GSH to protect from ferroptosis was later related to the optimal activity of the enzyme glutathione peroxidase 4 (GPX4), a selenoprotein required for the efficient reduction of peroxidized phospholipids^{3–5} and to suppress the activation of arachidonic acid (AA)-metabolizing enzymes⁶, which may contribute to the process of phospholipid peroxidation. Since then, a complex interplay between lipid, iron and cysteine metabolism has emerged as an important regulator of this cell death pathway.

In the majority of cells in culture, sensitivity to ferroptosis is dictated by the presence of acyl-CoA synthetase long chain family member 4 (ACSL4), an enzyme responsible for the esterification of polyunsaturated fatty acids (PUFAs) to acyl-CoA, a required step for the formation of PUFA-containing phospholipids^{7–9}. How exactly oxidation of PUFAs above a certain threshold drives cell death is, however, currently unknown: it might be caused by the rupture of the plasma membrane in response to the accumulation of truncated phospholipid species or potentially by the inactivation of critical pro-survival proteins by lipid-derived electrophiles originating from lipid peroxidation^{10,11}. Additionally, cystine supply through the activity of the cystine–glutamate antiporter (system xc-) or the transsulfuration pathway has been recognized as a critical upstream process of ferroptosis^{12–14} (FIG. 1). Further studies

have also put forward the importance of transferrin trafficking and ferritin degradation through the process of ferritinophagy, a specific form of autophagy required for the degradation of ferritin, as critical determinants of ferroptosis sensitivity via an increase in the so-called labile iron pool^{15–18}. Despite the general importance of this pathway to sustaining cell survival in non-cancerous cells and tissues¹⁹, it has been demonstrated that several oncogenic pathways render cancer cells extremely susceptible to this form of cell death through the modulation of key regulatory ferroptosis checkpoints^{20–24}. The recognition that oncogenic signalling generates an inherent ferroptosis sensitivity puts forward a curious dilemma: how can the downregulation of mechanisms that suppress ferroptosis, associated with an increase in the steady-state levels of lipid peroxidation, provide a growth or survival advantage to cancer cells?

Spurred by this apparent paradox, in this Opinion article, we present a discussion of the recent advances in the understanding of ferroptotic processes and offer a perspective on how the modulation of key elements regulating ferroptosis sensitivity could ultimately impinge on the interactions of cancer cells with the immune compartment. We provide a GPX4-centric view of how the modulation of key metabolic determinants that ultimately impact on the activity of this critical pro-survival protein controls relevant events required for cancer growth and persistence. We also discuss the related discovery of the involvement of AA metabolism in immune evasion and propose that AA-derived oxidation products generated during ferroptosis or by ferroptosis-sensitive cells could lead to a similar outcome. We believe this knowledge should guide us in assessing the benefits and potential pitfalls of triggering ferroptosis in the context of cancer treatment.

Regulation of ferroptosis by GPX4

GPXs are a family of enzymes that reduce hydroperoxides at the expense of the oxidation of two molecules of GSH. The optimal activity of these enzymes is thus directly linked to GSH metabolism, whereby a decrease in cysteine–GSH metabolism ultimately suppresses

Glossary

 β -Oxidation

A catabolic process in which fatty acid molecules are oxidized in mitochondria to generate acetyl-CoA, NADH and FADH, the last two of which drive forces of the electron transport chain.

Conventional type 1 dendritic cell

(cDC1). A subset of dendritic cells dependent on the transcription factor BATF3 for development and characterized by the specific expression of C-type lectin receptor DNGR1.

Eicosanoids

Bioactive metabolites derived from the enzymatic and non-enzymatic oxidation of arachidonic acid and other polyunsaturated fatty acids that are 20 carbon units in length.

Labile iron pool

A transient pool of chelatable redox-active iron.

Mevalonate pathway

A metabolic pathway responsible for the generation of sterol isoprenoids, such as cholesterol, and non-sterol isoprenoids including dolichol, haem A, isopentenyl tRNA and ubiquinone.

Necrotic cell death

In contrast to the prototype of programmed cell death, that is, apoptosis, this umbrella term is used to identify cells that share similar terminal features that include, but are not limited to, extracellular extravasation and immunogenicity.

Peroxidatic cysteine

A cysteine residue found in the catalytic site of several redoxins that is responsible for the nucleophilic attack of a peroxide bond.

Transsulfuration pathway

A metabolic pathway responsible for the interconversion of methionine to cysteine.

GPX activity. The GPX family consists of eight distinct members in mammals. GPX1, GPX2, GPX3 and GPX4 are selenoproteins harbouring a selenocysteine (Sec) in their catalytic centres, and GPX6 is a selenoprotein only in humans, whereas all others use a peroxidatic cysteine in their active site²⁵. All GPXs work as dedicated enzymes responsible for the detoxification of H₂O₂ and organic peroxides. Unlike other GPXs, GPX4 has a broader substrate preference, being the only enzyme so far described able to directly reduce complex phospholipid hydroperoxides²⁶. This unique activity of GPX4 is likely why it is the sole GPX essential for embryogenesis²⁷. It has also been recently demonstrated that several cancer states driven by oncogenic mutations and through stem cell-like or dedifferentiated states converge on an exquisite metabolic state addicted to GPX4 activity²⁸. Thereby, proper functioning of GPX4 appears to be critical for cell survival, as it is responsible for the efficient removal of phospholipid hydroperoxides. Phospholipid hydroperoxides, if not efficiently quenched by GPX4, are able to trigger a catalytic reaction in the presence of transition metals such as iron that eventually causes cell death²⁹. GPX4 has also been proposed to contribute to other cell death modalities, and some (early) reports have linked GPX4 activity to sensitivity to apoptosis³⁰, necroptosis³¹ and pyroptosis³². Yet it remains unclear whether these cell

death pathways indeed share common features³³ or whether the occurrence of lipid hydroperoxides just sensitizes to cell death stimuli. Hence, it remains puzzling why certain oncogenic signalling allows cancer cells to exist in such a vulnerable state. Or perhaps there are still yet to be discovered benefits arising from such an acquired vulnerability for tumour persistence.

Ferroptosis regulation in cancer

Mechanisms underlying susceptibility and resistance to ferroptosis particularly in the context of cancer have been an intense area of research in the past few years. Although it is well accepted that ferroptosis execution requires the oxidation of PUFAs, the underlying mechanisms of how oncogenic mutations and other non-oncogenic cancer-relevant states sensitize to ferroptosis have started to emerge but remain largely uncharacterized. In accordance with this concept, several potential mechanisms to target this vulnerable state have been put forward and are briefly discussed in BOX 1.

Tumour suppressors control ferroptosis sensitivity. The tumour suppressor p53, also referred to as the 'guardian of the genome', which is mutated in approximately 50% of all cancers³⁴, was the first to be linked to increased sensitivity to ferroptosis. Initial associations between p53 and ferroptosis came from studies using a mouse model in which three lysines (K117R, K161R and

K162R) in the p53 DNA binding site are simultaneously mutated (p53-3KR mice). Cells in p53-3KR mice, while not able to undergo apoptosis or cell cycle arrest, are still resistant to spontaneous tumorigenesis by a mechanism that requires metabolic adaptation³⁵. Further characterization of these animals demonstrated that the metabolic alteration required to suppress tumorigenesis was the inhibition of transcription of *Slc7a11*, which encodes the substrate-specific subunit of system xc⁻²² (FIG. 1). Repression of SLC7A11 was sufficient to sensitize cancer cells to an oxidant insult, rendering cells prone to undergo a ferroptosis-like cell death. Moreover, the same group identified a fourth acetylation site (K98) in p53, which, when concomitantly mutated with the other previously described acetylation sites, generated a p53 variant that was no longer able to bind to the promoter of *Slc7a11*. Unlike the p53-3KR mice, these mice developed tumours like those in p53 null mice³⁶. This study thus draws a link between the antitumorigenic function of p53 and ferroptosis through the specific binding of p53 to the promoter of *Slc7a11*. Subsequently, another group has demonstrated that a common single nucleotide polymorphism found in p53 in individuals of African descent, which yields the mutant form P47S, is associated with an increased incidence of cancer in mouse models, and cells carrying this mutation are resistant to ferroptosis via altered glutamine metabolism³⁷.

If indeed increased sensitivity to ferroptosis is a major contributor to tumour suppression by p53, an additional question arises: what would be the physiologically relevant stressor that ultimately sparks the ferroptosis cascade? For now, it can be speculated that potential candidates could be H₂O₂ generated by natural killer (NK) cells through NADPH oxidase 2 (NOX2)³⁸. Another potential critical function of p53 in regulating ferroptosis might be based on its recent recognition as a key mediator of the mevalonate pathway. Under metabolic stress, p53 mediates the expression of ATP-binding cassette subfamily A member 1 (ABCA1). ABCA1 is then responsible for the retrotranslocation of cholesterol from the plasma membrane to the endoplasmic reticulum, leading to the inactivation of sterol regulatory element binding protein 2 (SREBP2)³⁹. Inhibition of SREBP2 causes an overall suppression of transcription of genes in the mevalonate pathway, ultimately suppressing the production of several metabolites, such as squalene and

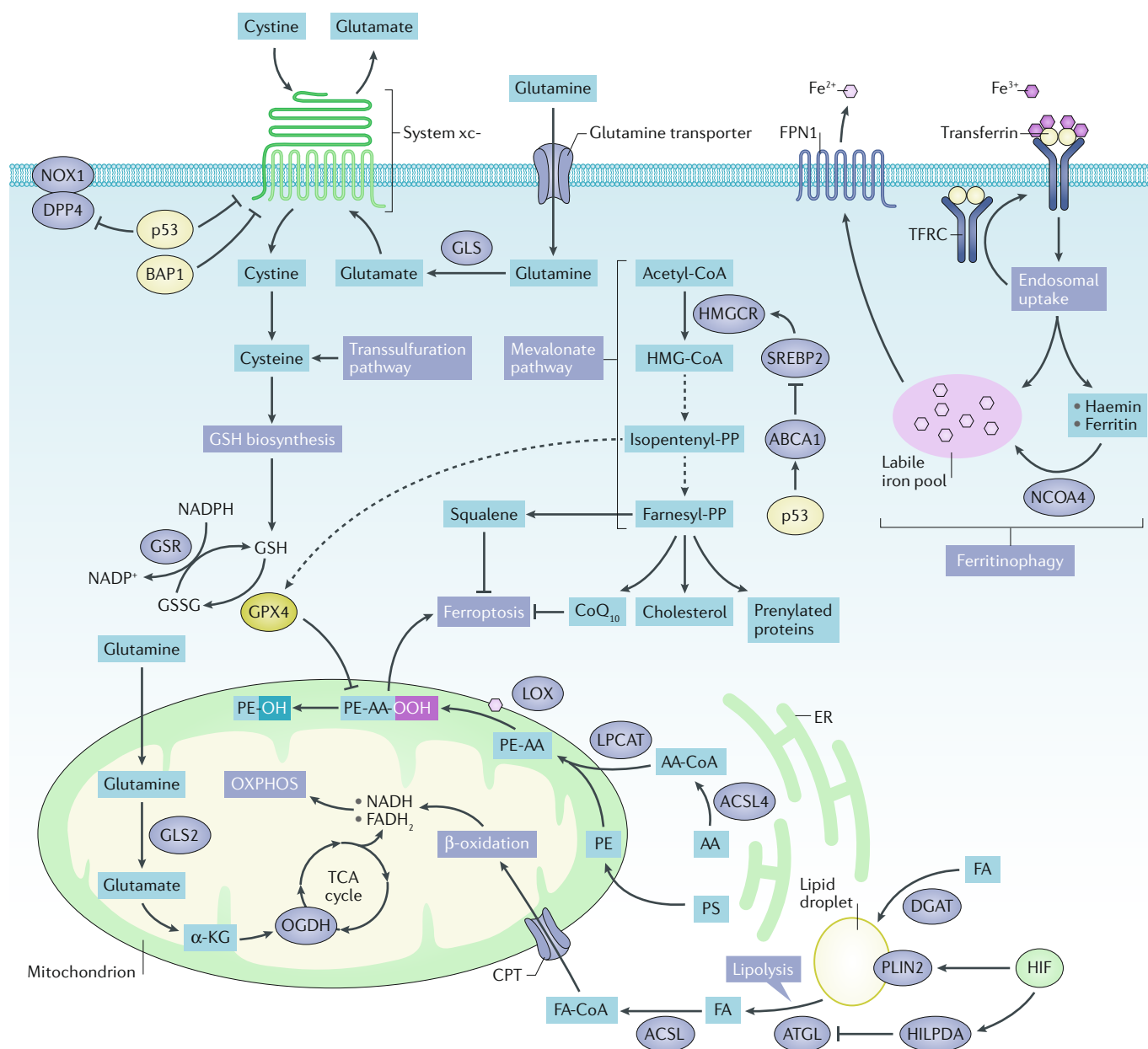


Fig. 1 | The main metabolic processes regulating ferroptosis and GPX4 activity. The key ferroptosis regulator glutathione peroxidase 4 (GPX4) and upstream events modulating sensitivity are shown. Cysteine and glutathione (reduced) (GSH) availability supported by cystine uptake or the transsulfuration pathway is at the core of ferroptosis by providing reducing equivalents for the optimal function of GPX4 (REFS^{3,4}). This has been recently recognized as an important checkpoint controlled by the known tumour suppressors BRCA1-associated protein 1 (BAP1)⁴⁷ and p53 (REF²²), generating an intrinsic sensitivity to ferroptosis. The mevalonate pathway is involved in ferroptosis by generating a series of biomolecules with potential anti-ferroptotic activity such as squalene⁴¹, ubiquinone⁴⁰ and isopentenyl-pyrophosphate (PP)²¹, the last of which stabilizes the selenocysteine-specific tRNA required for efficient GPX4 translation. Iron uptake via the transferrin receptor or degradation of ferritin^{16,17} iron stores increases the labile iron pool, thereby sensitizing cells to ferroptosis via facilitation of a Fenton-like reaction of pre-formed lipid hydroperoxides. Iron uptake and processing, acyl-CoA synthetase long chain family member 4 (ACSL4)-dependent shaping of the cellular phospholipidome⁷ and the tricarboxylic acid (TCA) cycle are additional cellular processes that may contribute to ferroptosis sensitization. Recently, the

impact of the hypoxia-inducible factor (HIF) system on fatty acid (FA) metabolism has been appreciated²⁰, and the mobilization of lipid from droplets leads to modulation of ferroptosis sensitivity, depending on the FA composition of the latter. Additionally, channelling of polyunsaturated fatty acids (PUFAs) for catabolic purposes lowers their incorporation into phospholipids, thus decreasing sensitivity to ferroptosis^{6,20}. α -KG, α -ketoglutarate; AA, arachidonic acid; ABCA1, ATP-binding cassette subfamily A member 1; ATGL, adipose triglyceride lipase (also known as PNPLA2); CoQ₁₀, coenzyme Q₁₀; CPT, carnitine palmitoyltransferase; DGAT, diacylglycerol O-acyltransferase; DPP4, dipeptidyl peptidase 4; ER, endoplasmic reticulum; FPN1, ferroportin 1 (also known as SLC40A1); GLS, glutaminase; GSR, glutathione disulfide reductase; GSSG, glutathione disulfide; HILPDA, hypoxia-inducible lipid droplet-associated; HMGCR, HMG-CoA reductase; LOX, lipoxygenase; LPCAT, lyso-phosphatidylcholine acyltransferase; NCOA4, nuclear receptor co-activator 4; NOX1, NADPH oxidase 1; OGDH, oxoglutarate dehydrogenase; OXPHOS, oxidative phosphorylation; PE, phosphatidylethanolamine; PLIN2, perilipin 2; PS, phosphatidylserine; SREBP2, sterol regulatory element binding protein 2; system xc-, cystine–glutamate antiporter; TFRC, transferrin receptor.

Box 1 | Triggering ferroptosis — a novel approach in experimental cancer therapy

Ferroptosis was discovered in a high-throughput screen for molecules selectively inducing death in cancer cells carrying an oncogenic form of RAS², suggesting that ferroptosis is a promising alternative cell death modality to be exploited for cancer treatment. Currently, several strategies to target ferroptosis have been put forward and comprise the direct or indirect inhibition of the cystine–glutamate antiporter (system xc⁻; for example, erastin², sorafenib¹¹² and sulfasalazine¹¹³), inhibition of glutathione (reduced) (GSH) synthesis through γ -glutamylcysteine synthetase inhibition (for example, L-buthionine sulfoximine⁷⁴) or inhibition of glutathione peroxidase 4 (GPX4; for example, RSL3 (REF.⁴), ML210 (REF.²¹), withaferin A¹⁰² and FIN56 (REF.⁴⁰)). As stated, some of these molecules are direct inhibitors, while others have less characterized modes of action that appear to destabilize critical ferroptosis checkpoints via yet poorly characterized mechanisms. Notwithstanding, ferroptosis can be elicited in various mouse tumour models including a diffuse large B cell lymphoma xenograft model¹¹⁴ and in human cancer cell lines, such as melanoma²³, breast^{115,116}, gastric¹¹⁷, neuroblastoma¹⁰² and ovarian carcinoma⁴⁴ cell lines.

Moreover, recent studies indicate that some of the well-known chemotherapeutics, such as cisplatin¹¹⁸ and doxorubicin¹¹⁹, are capable of inducing ferroptosis-like cell death, urging for a careful re-evaluation of chemotherapy and combinatorial strategies regarding the type of cell death they induce. Experimental strategies to increase the efficiency of chemotherapeutics have also highlighted the value of nanomaterials, which can be used as a strategy to increase the efficiency of drug conjugates and to decrease systemic side effects. In this regard, several studies showed that packaging chemotherapeutics into nanocarriers is an efficient approach to induce ferroptosis in cancer cells *in vitro* and, if applied *in vivo*, leads to tumour eradication in mice. For example, doxorubicin packed into mesoporous carbon nanoparticles induced ferroptosis in cancer cells¹²⁰. The nano-targeting of withaferin A, a natural ferroptosis-inducing agent, efficiently killed a heterogeneous panel of high-risk neuroblastoma cell lines and suppressed growth of neuroblastoma xenografts in mice¹⁰². Interestingly, nanoparticles themselves (for example, poly(butylcyanoacrylate) and zero-valent iron nanoparticles and arginine-rich manganese silicate nanobubbles) can induce ferroptosis in cancer cells^{121–123}, and the capability of induction of a specific cell death type is highly dependent on nanoparticle structure and chemical modifications¹²². Importantly, iron ionophores that sequester iron into lysosomes and stimulate ferritin degradation also induce ferroptosis-like cell death *in vivo*¹¹⁶, adding to the current arsenal of potential ways to elicit ferroptosis *in vivo*. Future studies should provide the necessary insights for the validation of ferroptosis inducers in real clinical settings.

mono-deubiquitylates histone H2A, thereby directly affecting gene expression. Several genes that responded to BAP1-mediated H2A mono-deubiquitylation (H2A–ub) were intriguingly related to cellular metabolism. Among the most downregulated genes, the authors identified *SLC7A11*, and this downregulation was associated with a higher sensitivity towards ferroptotic stimuli. Yet inhibition of *SLC7A11* appears to intersect with different tumour suppressor mechanisms leading to an increased sensitivity to ferroptosis. It is also worth mentioning that the characterization of BAP1-deficient mice has recently unravelled that its loss markedly upregulates mevalonate pathway genes and alters lipid metabolism, which ultimately hints at similar metabolic adaptations to those described for p53 (REF.⁴⁸).

These shared similarities impinging on cysteine metabolism and the mevalonate pathway driven by disparate oncogenic drivers support the notion that some cancers converge on this phenotype and thus might share inherent dependency on GPX4 activity. Hence, these findings should stimulate further research to assess whether these features, including impaired cystine uptake and decreased intermediates of the mevalonate pathway, are present in other tumour types and involve other tumour suppressors.

Hypoxia-inducible factors and ferroptosis sensitivity.

Initial reports on the sensitivity of cancer cell lines to ferroptosis inhibition have indicated a strong dependence on GPX4 in renal cell carcinomas (RCCs)⁴, but the molecular underpinnings of this dependency have started to emerge only recently. In clear cell RCC (ccRCC), the most common type of RCC, loss of the von Hippel–Lindau (*VHL*) gene markedly renders this type of tumour sensitive to GSH depletion-induced ferroptosis, which was partially associated with a decrease in β -oxidation²⁰. Curiously, the same authors²⁰ and another group⁴⁹ have identified a marked increase in arachidonate 5-lipoxygenase (*ALOX5*) in *VHL*-deficient ccRCC cells, pointing towards a stronger inflammatory potential mediated by the formation of *ALOX5*-dependent eicosanoids by this type of cancer⁴⁹. Recent studies suggest that the hypoxia-inducible factor (HIF) pathway is a key driver of this vulnerability⁵⁰. Thereby, HIF2 α was shown to stimulate the specific enrichment of PUFAs in an *ACSL4*-dependent manner in ccRCC. The authors further identified *PTGS2*, which encodes prostaglandin-endoperoxide synthase 2, as being among

ubiquinone, that have been associated with ferroptosis suppression^{40,41}. As such, blocking the mevalonate pathway, and assuming the intermediates are not directly obtained from the extracellular space, could lead to an inherent state of ferroptosis sensitivity. Moreover, studies using a mouse model of lung tumorigenesis driven by KRAS-G12D and mutant p53 unravelled that the p53-R270H mutant has a dominant negative effect on wild-type p53 leading to the upregulation SREBP2 target genes, which was associated with an increased sensitivity to HMG-CoA reductase inhibition by statins⁴². This is reminiscent of recent reports showing that ferroptosis-sensitive cells are also sensitive to statins²¹, ultimately suggesting that the p53-R270H mutant, at least in this lung cancer model, could benefit from strategies targeting ferroptosis⁴².

Recent reports have pointed to an even more complex picture, where, in some conditions, wild-type p53 might provide a pro-survival advantage by, curiously, boosting antioxidant defence. p53-mediated activation of p21 (encoded by *Cdkn1a*) was shown to suppress phospholipid oxidation by preserving intracellular thiols, including GSH⁴³. This is in line with an earlier report

showing that, under metabolic stress, caused by serine starvation, cancer cells activate the p53–p21 axis to preserve GSH levels through redirecting serine used for nucleotide synthesis to support GSH levels⁴⁴. Another potential anti-ferroptotic function of p53 has been postulated and involves the sequestration of dipeptidyl peptidase 4 (DPP4); if the NOX1–DPP4 association at the plasma membrane is prevented, ferroptosis is suppressed⁴⁵. Hence, it seems that p53 phenotypes are likely to be tumour type-specific, enforcing the notion that the implication of p53 in ferroptosis needs to be rationalized in a tissue-dependent and context-dependent manner.

In addition to p53, the tumour suppressor BRCA1-associated protein 1 (BAP1), loss of function mutations of which have been associated with several human cancers including mesotheliomas (36–65%), uveal melanomas (32–47%), cholangiocarcinomas (20–30%) and clear cell renal cell carcinomas (ccRCCs; 10–15%)⁴⁶, has recently been associated with ferroptosis⁴⁷. BAP1 is a predominantly nuclear-located deubiquitinase (DUB) that is responsible for the formation of the polycomb repressive deubiquitinase (PR-DUB) complex that

the most upregulated genes by HIF2 α . This tentatively accounts for the potential of this tumour type to evade immune surveillance via increased production of intratumoural prostaglandin E₂ (PGE₂) (see also below). It is worth mentioning that in a recent study the DNA methylation modifier lymphoid-specific helicase (HELLS) was reported to inhibit ferroptosis by activating lipid metabolism-associated genes via inhibition of prolyl hydroxylase domain-containing protein 2 (PHD2; also known as EGLN1) and stabilization of HIF1 α ⁵¹. Therefore, it will be relevant in the future to dissect the relative contribution and potential overlapping effects of HIF transcription factors in ferroptosis. Along the same line, glutamate released via system xc⁻ was reported to act as a paracrine signal by activating HIF1 α in breast cancers. Mechanistically, the extracellular excess of glutamate is sufficient to retro-inhibit system xc⁻, leading to reduced intracellular cysteine levels, which may account for the observed increased oxidation of PHD2 (REF.⁵²). Thus, the high expression of SCL7A11 found in tumours such as triple-negative breast cancer (TNBC)⁵³ could be associated with the enhanced activation of HIF1 α and HIF2 α . It has also been reported that activation of HIF1 α and HIF2 α in TNBC is achieved through an increase in the steady-state levels of the endoplasmic reticulum-induced X-box binding protein 1 (XBP1) as a response to loss of the oestrogen receptor⁵⁴. Interestingly, these data are in line with the finding that TNBC cells are specifically enriched with PUFAs through ACSL4-dependent activity⁷. Consequently, ccRCC and TNBC appear to converge, through different signalling mechanisms, on a similar metabolic node that is the ACSL4-dependent PUFA-enriched state marked by the presence of lipid droplets. This lipid-enriched state through the accumulation of lipid droplets ultimately has been recognized as a reservoir for AA or other PUFAs that are mobilized not only when nutrients become scarce but also for the formation of signalling lipid mediators, such as eicosanoids⁵⁵. It is also worth mentioning that, at least in astrocytes, lipid droplets could provide a survival advantage by acting as a sink for lipid hydroperoxides⁵⁶.

Mesenchymal-like states and ferroptosis sensitivity. Recently, non-mutational states associated with a mesenchymal-like phenotype and highly resistant to standard therapy have been associated with ferroptosis sensitivity. Several independent

reports have shed light on similar states adopted by cancer cells that are linked to an inherent sensitivity to ferroptosis. A systematic exploration of the cellular response to ferroptosis-inducing small molecules uncovered a genetic signature consistent with a high-mesenchymal state driven by zinc-finger E-box binding homeobox 1 (ZEB1)²¹. Similar features were recently reported in a broader panel of cancer cell lines, which identified a GPX4 dependency in cells having an epithelial-mesenchymal transition gene signature⁵⁷. Moreover, treatment of melanoma, breast cancer and lung cancer cell lines with first-line chemotherapeutic agents leaves a population of residual cancer cells, so-called persister cells, which have cancer stem cell-like features as well as a mesenchymal-like gene expression signature⁵⁸. A mesenchymal state has been associated with the development of resistance to other cancer therapies, such as targeted BRAF inhibitors⁵⁹. Similarly, melanoma cells can adopt different states along a differentiation trajectory, whereby more differentiated melanomas respond well to small molecules targeting mutant BRAF, such as vemurafenib²³. Curiously, vemurafenib-induced dedifferentiation increases sensitivity to ferroptosis, and this dedifferentiated state is also associated with immune evasion owing to the lack of melanocyte-specific antigens expressed by melanoma cells²³. Independent reports have also characterized marked lipidomic changes, including the accumulation of PUFAs, in melanomas treated with vemurafenib⁶⁰. In this specific setting, it would be important to assess how crucial enzymes of the ferroptosis cascade, such as ACSL4, are modulated, as this could ultimately provide the missing mechanistic link for how sensitivity to ferroptosis is acquired.

Despite our still incomplete understanding of what drives these poorly differentiated states and associated ferroptosis sensitivity, some mechanistic cues are surfacing. As noted above, Viswanathan et al.²¹ made a compelling case for the involvement of ZEB1, a transcription factor reported to enhance stemness, colonization capacity and metabolic plasticity of tumour cells⁶¹. Interestingly, ZEB1 has also been reported to be a central regulator of lipid metabolism and adipogenic fate both in vivo and in vitro⁶². The underlying mechanism of this phenotypic outcome, which overlaps with the dependency of GPX4, remains to be clarified but might rest on the complex lipidomic remodelling driven by ZEB1. Of note, alternative drivers of stem cell-like

features that sensitize to ferroptosis cannot be excluded. Thus, more work will be required to define the exact contribution of ZEB1 in different models and to firmly link stemness with increased sensitivity to ferroptosis.

GPX4 and inflammation

The notion that a chronic inflammatory state promotes and supports tumour growth and metastasis is generally accepted, and a chronic inflammatory state has been long associated with increased incidence of a series of malignancies, which include but which are not limited to oesophageal, gastric, hepatic, pancreatic and colorectal cancers⁶³. Moreover, several of the oncogenic mutations and metabolic states described above have been shown to converge on an inflammatory phenotype^{64–66}. In simple terms, chronic inflammation can be defined as the absence of resolution of the initial inflammatory process through loss of the finely tuned regulation by a series of pro-inflammatory and anti-inflammatory molecules, such as lipid-derived mediators⁶⁷. In this context, early reports on the activity of GPX4 being required to suppress the metabolism of AA to generate eicosanoids^{68–71} are particularly informative to rationalize how the downregulation of the GSH–GPX4 axis and an increased level of phospholipid peroxidation could benefit the cancer cell (FIG. 2).

Specifically, eicosanoid biosynthesis is initiated by the stereospecific oxygenation of AA catalysed by specific dioxygenases, either cyclooxygenases (COXs) or lipoxygenases (LOXs), yielding hydroperoxyeicosatetraenoic acid (HPETE)

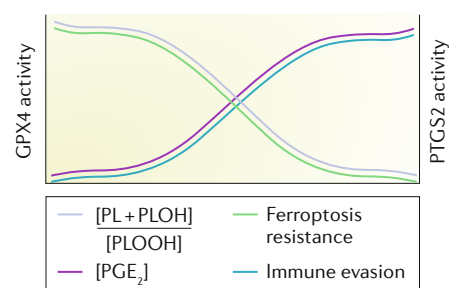


Fig. 2 | Hypothetical model of an inverse correlation of the cellular peroxide tone and immune evasion. Because the activity of prostaglandin-endoperoxide synthase 2 (PTGS2) is directly regulated through the cellular peroxide tone ([PL + PLOH]/[PLOOH]) via glutathione peroxidase 4 (GPX4), prostaglandin E₂ (PGE₂)-mediated tumour immune evasion is most likely linked to an acquired ferroptosis sensitivity. PL, phospholipid; PLOH, phospholipid alcohol; PLOOH, phospholipid hydroperoxide.

and prostaglandin G₂ as intermediates, respectively. Activity of these enzymes is under direct control of the intracellular redox state, the so-called peroxide tone⁷². The peroxide tone refers to the steady-state level of cellular lipid hydroperoxides that is ultimately required for the activation of both LOXs and COXs⁷³. Consequently, cysteine metabolism and GSH–GPX4 activity directly suppress the activation of these enzymes through the decrease in the intracellular peroxide tone^{74,75}. Under permissive conditions, AA can be metabolized to different eicosanoids through the action of the COX pathway (which generates prostanoids, including PGE₂ and thromboxanes) or the LOX pathway (which produces leukotrienes and hydroxyeicosatetraenoic acids (HETEs)). Moreover, AA can also be a substrate of the cytochrome P450 family of enzymes (which generates epoxyeicosatrienoic acids and HETEs). But because the activity of these enzymes is not directly linked to the intracellular peroxide tone, it is not expected that GPX4 would impact this branch of AA metabolism.

Importantly, eicosanoid-like molecules can also be generated in the absence of these enzymes through free radical-mediated lipid autooxidation catalysed by transition metals, ultimately leading to the formation of isoprostanes⁷⁶. In this regard, it has been reported that ferroptosis-sensitive cells, and cells undergoing ferroptosis, are characterized by the massive release of oxidized lipid mediators³, although the identity of these species has not been thoroughly characterized, particularly under different levels of GPX4 activity; therefore, this should be considered as an important area for further development. It is also conceivable that ferroptotic cancer cells could function as donors of AAs for transcellular biosynthesis of eicosanoids⁷⁷. Furthermore, through the cooperation with multiple different cell types present in the tumour microenvironment, transcellular biosynthesis might be involved in the generation of biologically active immunomodulatory AA metabolites that affect antitumour immunity. In this regard, it has been shown that LOXs secreted by bacteria could induce phospholipid oxidation in lung epithelial cells, causing ferroptosis in the host⁷⁸. It is thus tempting to speculate that similar mechanisms could occur during the crosstalk between cancer cells and cells of the immune compartment.

The concepts laid out above are important to reconcile how cell-autonomous functions can modulate intercellular

communication; however, circulating non-cell-autonomous factors, such as tumour necrosis factor (TNF), PGE₂, interleukin-6 (IL-6) and IL-1 β , must also be considered⁷⁹. In fact, some of these molecules have been shown to directly affect GPX4 levels and activity in cancer cells. It was shown that TNF treatment of cells leads to a sustained downregulation of GPX4 and that this is required for the generation of lipid mediators⁸⁰. TNF and IL-1 β are strong inducers of PTGS2, and chronic exposure to these pro-inflammatory cytokines is predicted to generate a high activity state of PTGS2, paired with low GPX4 activity. Supporting such an auto-amplification loop, in a model of chronic inflammation induced by a high-fat diet, *Gpx4* haploinsufficient mice presented a strong chronic inflammatory state characterized by an increase in TNF, inducible nitric oxide synthase (iNOS), IL-6 and IL-1 β ⁸¹. Unfortunately, the authors of this study did not explore further the role of specific lipid mediators. Ultimately, this concept might be an overall simplification, as the generation of eicosanoids from cells in an inflammatory-like state could be pro-inflammatory or anti-inflammatory depending on the oxidase and the lipid substrate, which calls for further studies aiming to shed light on the identity of these oxygenated lipid mediators in different cancer contexts.

It should be stressed that GPX4 activity is important for the survival not only of cancer cells but also of immune cells. In this regard, using genetically engineered mouse models, it has been shown that depletion of *Gpx4* in cells from the myeloid lineage increases septic lethality³². Knockout of *Gpx4* in the myeloid lineage also stimulated intestinal tumorigenesis via increased genetic instability in intestinal epithelial cells⁸². Further work on T cells has shown that antigen-specific CD8⁺ and CD4⁺ T cells lacking GPX4 failed to expand and were unable to protect host mice from acute lymphocytic choriomeningitis virus and *Leishmania major* parasite infections⁸³. These studies suggest an essential role of GPX4 in immunity and hint that modulation of GPX4 expression in certain immune cells will affect antitumour immunity. This should be a fruitful area for future research.

Immunomodulatory role of ferroptotic cancer cell death. It is known that dying cells, mostly in the context of apoptosis, communicate with the immune cells by a set of signals produced during cell death, such as ‘find me’ and ‘eat me’ signals⁸⁴. These signals allow immune cells to properly

locate dying (that is, apoptotic) cells in the tissue and mediate the movement of immune cells to and within tissues. Therefore, it is conceivable that, similar to apoptosis, ferroptotic cells will release distinct ‘find me’ signals, including lipid mediators, which will attract antigen-presenting cells (APCs) and other immune cells to the site of ferroptotically dying cells. Unlike apoptosis, it is currently unknown whether ferroptotic cancer cells indeed release such a set of signals. However, it has been recently shown that ferroptotic cancer cells are efficiently engulfed by macrophages in vitro, supporting the existence of such signals⁸⁵. Potential signals are AA oxidation products released from ferroptotic cells that may modulate antitumour immunity (FIG. 3). In this regard, it has been reported that ferroptotic cells release oxidized lipid mediators. Therefore, it is conceivable that LOXs, beyond their proposed role in oxygenating esterified PUFAs as ferroptotic signals, can also contribute to the release of immunomodulatory signals from ferroptotic cancer cells, thus affecting antitumour immunity. Ferroptotic cells have been shown to release eicosanoids such as 5-HETE, 11-HETE and 15-HETE in response to inducible GPX4 depletion³, whereas increasing GPX4 activity reduced pro-inflammatory lipid mediator production (5-HETE and LTB₄) and inhibited pro-inflammatory features driven by NF- κ B pathway activation in cells stimulated with TNF or IL-1 β ⁸⁶. Notably, LTB₄ is a well-established pro-inflammatory leukotriene with important roles in carcinogenesis⁸⁷.

Unlike the more established role of free eicosanoids as signalling molecules modulating immune response, much less has been uncovered for esterified eicosanoids, which are products derived by the direct action of lipoxygenases on sn-2 fatty acids in phospholipids or by the re-esterification of free eicosanoids into the phospholipid pool, despite the growing interest in their biological roles⁸⁸. Lipidomic analyses of cells undergoing ferroptosis have identified the accumulation of doubly and triply oxygenated AA-containing phosphatidylethanolamine (PE) species, possibly generated through the action of arachidonate 15-lipoxygenase (ALOX15)^{6,7,89}. The biological effects of extracellular oxidized PEs and products or their oxidative decomposition (so-called oxidatively truncated phospholipids) or hydrolysis (for example, lyso-phospholipids and oxygenated lyso-phospholipids) are mostly unknown (FIG. 3), but a role for

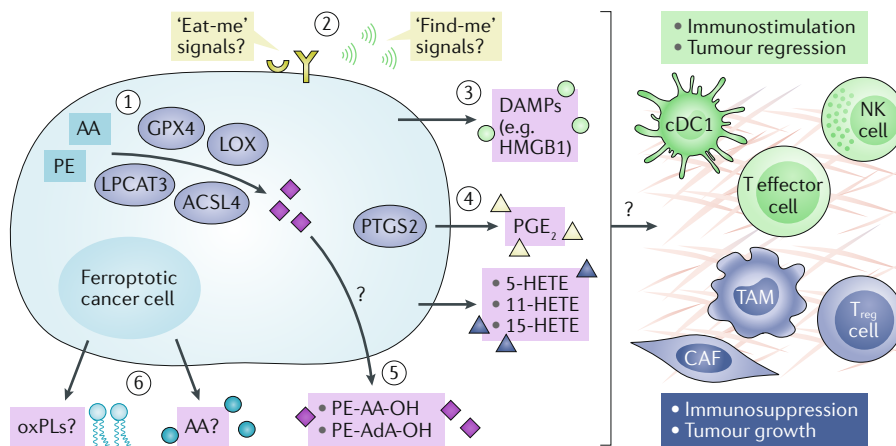


Fig. 3 | Possible modulation of tumour immunity by ferroptotic cancer cells. Sensitivity to ferroptosis is mediated by the activity of glutathione peroxidase 4 (GPX4), acyl-CoA synthetase long chain family member 4 (ACSL4), lyso-phosphatidylcholine acyltransferase 3 (LPCAT3) and lipoxygenases (LOXs) (labelled (1) in the figure). ACSL4 functions as a key regulator of ferroptosis execution, which catalyses esterification of arachidonic acid (AA) or adrenic acid (AdA) into phosphatidylethanolamine (PE), while LOXs may oxidize AA-PE and AdA-PE to support the formation of ferroptotic signals^{6,7}. It is known from apoptotic cell removal that clearance of dying cells in general is a complex process in which many surface molecules ('eat me' signals), adaptors (bridging molecules) and chemotactic molecules ('find me' signals) are involved and that is controlled at multiple levels (labelled (2) in the figure). Several lines of indirect evidence indicate that ferroptotic cells induce attraction and activation of innate immune cells (for example, neutrophils)^{3,101,103,128} and that they are efficiently engulfed by phagocytes⁹⁵. This suggests that find me signals might indeed be released during ferroptosis; however, at the moment, their nature is unknown. So far, high mobility group box 1 (HMGB1) (labelled (3) in the figure) is one of the known damage-associated molecular patterns (DAMPs) that was shown to be released during ferroptotic cell death⁹⁶. It is known that its release is required for immunogenicity of apoptotic cell death¹⁰⁵, pointing to the fact that ferroptotic cancer cells might indeed be immunogenic. It has been shown that ferroptotic cells also can release eicosanoids such as 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE and 15-HETE in response to inducible GPX4 depletion³ in addition to prostaglandin E₂ (PGE₂)⁴ (labelled (4) in the figure). Future studies will show whether molecules typically associated with ferroptotic cells (that is, PE-AA-OH and PE-AdA-OH (labelled (5) in the figure)) are involved in the modulation of the uptake of ferroptotic cells and whether they impact on antitumour immunity. It is also conceivable that potential immunomodulatory signals can be AA oxidation products and oxidized phospholipids (oxPLs) (labelled (6) in the figure), which might be released from ferroptotic cells, although additional studies are required to substantiate this. Therefore, various putative signals released by ferroptotic cancer cells may lead to the stimulation or suppression of different immune cells, thereby making ferroptotic cancer cells a double-edged sword in cancer. CAF, cancer-associated fibroblast; cDC1, conventional type 1 dendritic cell; NK, natural killer; PTGS2, prostaglandin-endoperoxide synthase 2; TAM, tumour-associated macrophage; T_{reg} cell, regulatory T cell.

lyso-phospholipids in the attraction of APCs has been previously demonstrated for apoptotic cells⁹⁰. It has been reported that ALOX15-derived lipid mediators regulate dendritic cell (DC) maturation and modulate adaptive immune responses. Specifically, oxidized phosphatidylcholine inhibited DC maturation via activation of the transcription factor NRF2 and dampened the differentiation of T helper 17 (T_H17) cells⁹¹.

Similarly, it has been shown that the efficiency of macrophage clearance of apoptotic cells can be modulated by the oxidative state of externalized phospholipids. This concept is supported by the fact that apoptotic cells that expose peroxidized phosphatidylserine (PS) at their outer

plasma membrane are more efficiently engulfed by macrophages than cells lacking this oxidative signal⁹². Of note, it has also been shown that oxidized PE is able to block the engulfment of apoptotic cells by mouse inflammatory macrophages in vitro and in vivo⁹³.

Moreover, oxidized lipids and lipid droplets have also been implicated in the regulation of antitumour immune responses. In the tumour microenvironment, DCs accumulate large amounts of oxygenated neutral lipids, and PUFA triacylglycerols and free PUFAs form large lipid droplets that lead to defective cross-presentation and defective antitumour immunity^{94,95}.

Ferroptotic cancer cells interact with the immune system at several steps, the

most crucial of which are phagocytosis and migration, maturation, antigen processing and cross-presentation by DCs. Each of these steps as detailed above could potentially be modulated by oxidized PL derived from ferroptotic cancer cells, but this link has not been experimentally addressed yet.

Beyond lipid mediators, it has been shown that different types of ferroptotic cancer cells can release high mobility group box 1 (HMGB1) in an autophagy-dependent manner^{96,97}. HMGB1 belongs to the family of intracellular molecules called damage-associated molecular patterns (DAMPs). Once released outside of the cells, they acquire immunostimulatory properties and function as adjuvants, thereby contributing to the activation of the innate and adaptive immune systems by binding to pattern recognition receptors. HMGB1 is one of the key elements required for immunogenicity of cancer cells⁹⁸. In this regard, it has been reported that RAGE (also known as AGER) is required for HMGB1-mediated TNF release in macrophages in response to ferroptotic cells⁹⁶. Although this study strongly suggests that ferroptotic cancer cells could be immunogenic in nature, similarly to necroptotic cancer cells^{99,100}, additional work is required to experimentally validate immunogenicity of ferroptotic cancer cells and to better understand their role in anticancer therapy. It will be equally important to address whether immunogenicity can be impacted by different ferroptosis-inducing strategies including nanoparticles, system xc⁻ inhibitors and GPX4 inhibitors. Several lines of indirect evidence in non-cancer models have associated ferroptosis with an increased attraction and activation of innate immune cells^{3,101,102}. In addition, in a model of cardiac injury, ferroptotic cells attracted neutrophils in a TLR4–TRIF-dependent manner¹⁰³, suggesting that DAMPs are released during ferroptotic cell death, which triggers TLR4 signalling. Of interest, a specific set of chemotherapeutics (for example, doxorubicin) can induce immunogenic apoptosis, which, similarly to ferroptotic cells, could induce attraction of neutrophils, and ferroptotically dying cells could be sensed by the innate immune system in a TLR4-dependent manner¹⁰⁴. It is known that during chemotherapy or radiotherapy, DCs require signalling through TLR4 for efficient processing and cross-presentation of antigens from tumour cells undergoing immunogenic apoptosis¹⁰⁵. Therefore, future studies will reveal whether TLR4 is required for the induction of the immune response

against ferroptotic tumour cells. Taken together, these results highlight the notion that ferroptotic cells can release such ‘find me’ signals.

Ferroptosis, prostaglandins and immune evasion. Prostaglandins, and more recently PGE₂, have attracted considerable attention as important immune modulators. Because it has been shown that induction of ferroptosis in cancer cells is associated with increased expression of PTGS2 and the release of PGE₂ (REF.⁴), it is conceivable that, if sufficient levels are achieved, a shift from antitumour to immunosuppressive responses might take place¹⁰⁶, leading to progressive tumour growth^{107,108}, although this requires experimental validation (FIG. 2). Interestingly, the release of PGE₂ occurs well before any cell death is noticeable, suggesting that toning down GPX4 activity is indeed sufficient to sustain a PTGS2-active state.

It has been shown that PGE₂ is a major immunosuppressive factor in a genetically well-defined cell line derived from a melanoma mouse model engineered to express mutant *Braf*^{V600E}, which is also the most common mutation found in patients. In this model, PGE₂ production was sufficient to blunt conventional type 1 dendritic cell (cDC1)-dependent CD8⁺ T cell-mediated immune control¹⁰⁷. Further work demonstrated that PGE₂ inhibits the infiltration of cDC1s into

the tumour site through the suppression of the chemokines CCL5 and XCL1 secreted by NK cells¹⁰⁸. The action of PGE₂ and its downstream signalling were not studied in great detail, but early work provided evidence of a strong immunosuppressive effect of PGE₂ towards NK cells¹⁰⁹. In addition to targeting NK cell activity, PGE₂ also hinders cDC1s directly by downregulating the chemokine receptors that promote recruitment into tumours. Therefore, PGE₂ impairs antitumour immunity by acting on at least two cell types of the innate immune system, NK cells and cDC1s.

In addition, PGE₂ also directly suppressed cytotoxic T cell action⁶³, underscoring its role as a major immunosuppressive mediator that interferes with multiple aspects of anticancer immunity. Recently, it was shown that, during chemotherapy cycles, PGE₂ is released from tumours and neighbouring cells and that this is critical for tumour repopulation by cancer stem cells, a process that was blocked by co-targeting PTGS2 (REF.¹¹⁰). It is thus tempting to speculate that tumours intrinsically sensitive to ferroptosis as part of a programme leading to decreased GPX4 activity could be prone to release more PGE₂. In fact, chemotherapy targeting fast cycling cells would ultimately select these mesenchymal-like states, which are then poised to generate PGE₂, thus ultimately enriching for a ferroptosis-sensitive cancer cell population. Moreover,

this provides an important consideration when triggering ferroptosis and the desired immunomodulatory effect^{88,93,111}. It is thus provocative to infer that, in the tumour bulk, a small proportion of cells undergoing ferroptosis might suppress the immune system and allow growth. Ultimately, the in-depth characterization of the profile of produced eicosanoids generated from AA (and other fatty acids) under different levels of GPX4 expression and activity will be of paramount importance to validate this hypothesis. This is relevant because specific sets of immunosuppressive eicosanoids are generated under partial loss of GPX4 activity³. Yet it is conceivable that, upon ferroptosis execution, these immunosuppressive eicosanoids are lost. Undeniably, more work is warranted to understand the immunomodulatory role of ferroptotic cancer cells in antitumour immunity.

Concluding remarks

Although substantial progress has been made in our understanding of how oncogenic states drive sensitivity to ferroptosis, much less is known about how this ultimately allows cancer cells to persist and proliferate. The notion proposed here is that this ferroptosis-sensitive state allows the cancer cells to generate lipid-derived mediators that are able to modulate intracellular and intercellular signalling pathways including receptor tyrosine kinase (RTK) signalling, leading to the growth of the cancer cell. Additionally, the complex interplay between different lipid oxidases (for example, LOXs and PTGS2) opens up new possibilities to combine and modulate them in the tumour site, thereby allowing an efficient immune response and potentiating the immunogenicity of ferroptotic cells. Hence, it is essential that we understand the role of these different oxygenated lipid mediators before we can explore the potential of inducing ferroptosis to efficiently kill tumour cells. Yet, one cannot neglect the idea that ferroptotic cancer cells might function as a double-edged sword, akin to the role of reactive oxygen species (ROS) and lipid ROS in RTK signalling (BOX 2), and if so, identifying the differences between ferroptosis that inhibits tumour growth and ferroptosis that drives cancer progression is crucial. To address this, it will be essential to better understand the molecular events controlling ferroptosis, which should help to identify methods that effectively kill cancer cells and at the same time prevent other cancer cells from escaping immune surveillance.

Box 2 | Lipid hydroperoxides and receptor tyrosine kinase signalling

The role of H₂O₂ in sustaining receptor tyrosine kinase (RTK) activation was reported over two decades ago and rests on the transient oxidative inactivation of key protein tyrosine phosphatases (PTPs) that normally target the phosphorylated form of the RTK¹²⁴. This concept led, among other features, to the establishment of a framework on how reactive oxygen species (ROS) stimulate tumour growth. Subsequently, much debate has been generated on which sources of ROS would be responsible for the sustained inactivation of PTPs. This led to the widespread acceptance that H₂O₂ derived from mitochondria as well as from professional H₂O₂-producing enzymes, such as NADPH oxidase 4 (NOX4), would be ultimately responsible for that. This view has, however, been criticized owing to the unfavourable reaction kinetics of H₂O₂ with PTPs, which afterward led to the concept that this oxidation might require a redox relay dependent on professional H₂O₂-removing enzymes, such as peroxiredoxins¹²⁵.

Much less attention has been given to alternative sources of oxidants; thus, it is critical to establish here that lipid hydroperoxides are also potential candidates to sustain RTK signalling via PTP inactivation. It has been reported that hydroperoxides generated by lipoxygenases are able to react at least three orders of magnitude more quickly with PTPs than H₂O₂, as exemplified by SRC homology region 2 domain-containing phosphatase 1 (SHP1) and PTP1 oxidation in the presence of 15-hydroperoxyeicosatetraenoic acid (15-HPETE)¹²⁶. This was further corroborated by the observation that *Gpx4* null cells show reduced levels of mature platelet-derived growth factor receptor-β (PDGFRβ) surface expression, increased steady-state PDGFRβ phosphorylation and reduced activity of PDGFRβ-targeting PTPs, effects that can be blunted by lipoxygenase inhibitors¹²⁶. These findings are in agreement with studies showing that the stable overexpression of glutathione peroxidase 4 (GPX4) is sufficient to slow down the G1 to S transition in a subset of cancer cells¹²⁷. Therefore, it is reasonable to assume that modulation of the cellular peroxide tone, which is determined by the steady-state level of lipid hydroperoxides in cells, will positively influence important features relevant for cancer proliferation.

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