OPINION

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

José Pedro Friedmann Angeli, Dmitri V. Krysko and Marcus Conrad

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 mechanism[1]. 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 death[2]. 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 enzymes[6], 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 tissues[19], 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 site[25]. All GPXs work as dedicated enzymes responsible for the detoxification of H_2O_2 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 hydroperoxides[26]. This unique activity of GPX4 is likely why it is the sole GPX essential for embryogenesis[27]. 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 activity[28]. 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 death[29]. GPX4 has also been proposed to contribute to other cell death modalities, and some (early) reports have linked GPX4 activity to sensitivity to apoptosis[30], necroptosis[31] and pyroptosis[32]. Yet it remains unclear whether these cell death pathways indeed share common features[33] 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 cancers[34], 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 adaptation[35]. 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 mice[36]. 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 metabolism[37].

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 H_2O_2 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

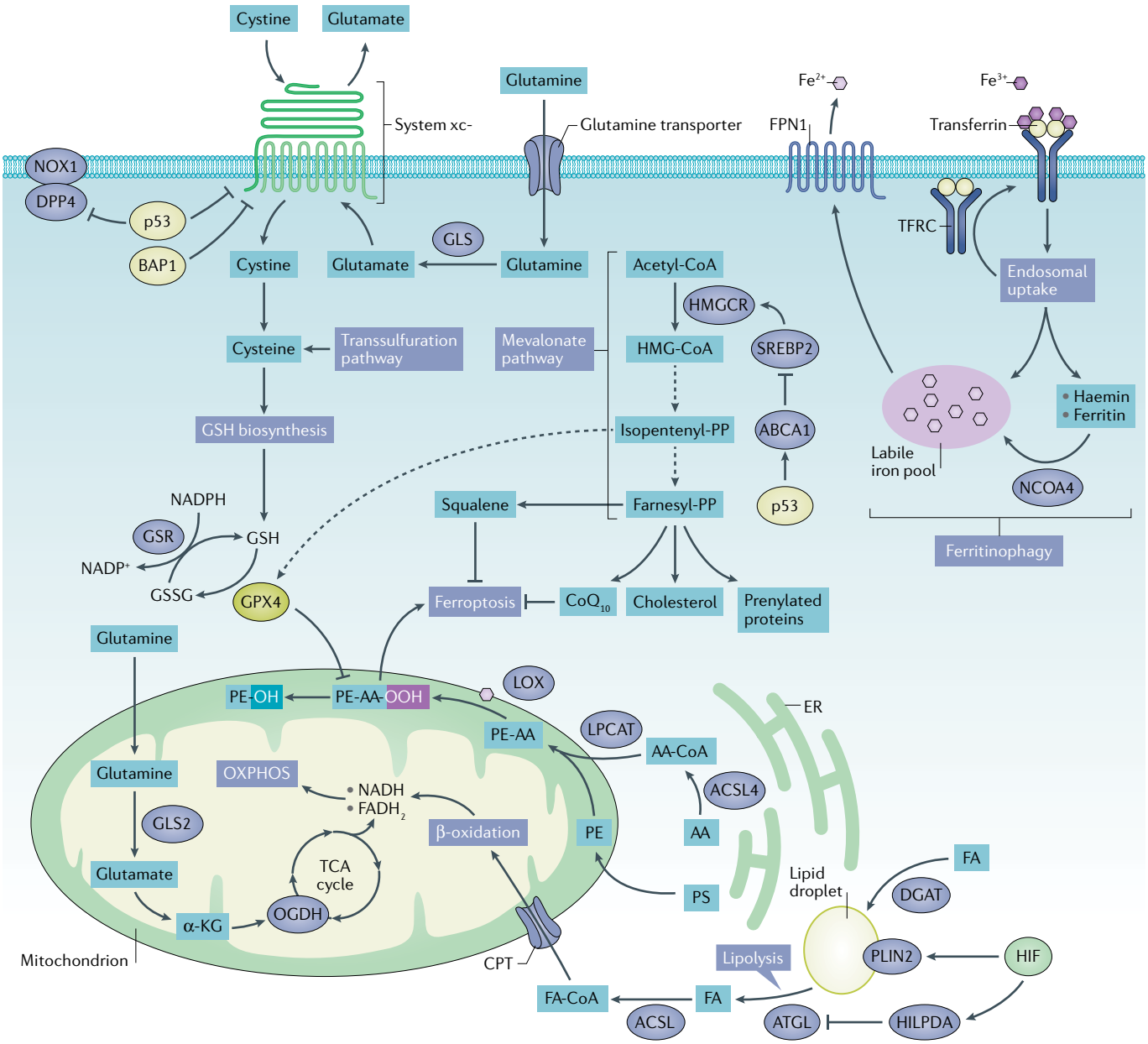

Fig. 1 | **The main metabolic processes regulating ferroptosis and GPX4 activity.** The key ferroptosis regulator glutathione peroxidase 4 (GPX4) and upstream events modulating sensitivity are shown. Cysteine and glutathione (reduced) (GSH) availability supported by cystine uptake or the transsulfuration 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 (REF.[22]), 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 squalene[41], ubiquinone[40] 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 phospholipidome[7] 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 appreciated[20], 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); CoQ_{10}, coenzyme Q_{10}; 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 RAS[2], 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, erastin[2], sorafenib[112] and sulfasalazine[113]), inhibition of glutathione (reduced) (GSH) synthesis through γ-glutamylcysteine synthetase inhibition (for example, L-buthionine sulfoximine[74]) or inhibition of glutathione peroxidase 4 (GPX4; for example, RSL3 (REF.[4]), ML210 (REF.[21]), withaferin A[102] 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 model[114] and in human cancer cell lines, such as melanoma[23], breast[115,116], gastric[117], neuroblastoma[102] and ovarian carcinoma[24] cell lines.

Moreover, recent studies indicate that some of the well-known chemotherapeutics, such as cisplatin[118] and doxorubicin[119], 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 cells[120]. 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 mice[102]. 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 modifications[122]. 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.

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 statins[42]. This is reminiscent of recent reports showing that ferroptosis-sensitive cells are also sensitive to statins[21], ultimately suggesting that the p53-R270H mutant, at least in this lung cancer model, could benefit from strategies targeting ferroptosis[42].

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 GSH[43]. 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 levels[44]. 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 suppressed[45]. 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 ferroptosis[47]. BAP1 is a predominantly nuclear-located deubiquitinase (DUB) that is responsible for the formation of the polycomb repressive deubiquitinase (PR-DUB) complex that 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-oxidation[20]. Curiously, the same authors[20] and another group[49] 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 cancer[49]. Recent studies suggest that the hypoxia-inducible factor (HIF) pathway is a key driver of this vulnerability[50]. Thereby, HIF2α 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

the most upregulated genes by HIF2α. This tentatively accounts for the potential of this tumour type to evade immune surveillance via increased production of intratumoural prostaglandin E_2 (PGE_2) (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 HIF1α[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 HIF1α 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 HIF1α and HIF2α. It has also been reported that activation of HIF1α and HIF2α 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 receptor[54]. Interestingly, these data are in line with the finding that TNBC cells are specifically enriched with PUFAs through ACSL4-dependent activity[7]. 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 eicosanoids[55]. It is also worth mentioning that, at least in astrocytes, lipid droplets could provide a survival advantage by acting as a sink for lipid hydroperoxides[56].

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 signature[57]. 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 signature[58]. A mesenchymal state has been associated with the development of resistance to other cancer therapies, such as targeted BRAF inhibitors[59]. 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 vemurafenib[23]. 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 cells[23]. Independent reports have also characterized marked lipidomic changes, including the accumulation of PUFAs, in melanomas treated with vemurafenib[60]. 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 cells[61]. Interestingly, ZEB1 has also been reported to be a central regulator of lipid metabolism and adipogenic fate both in vivo and in vitro[62]. 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 cancers[63]. 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 mediators[67]. 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)

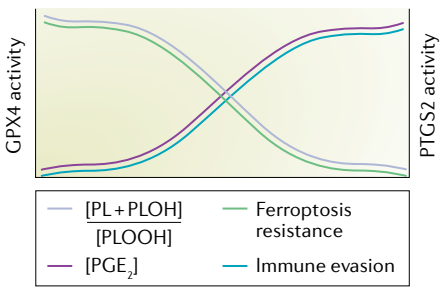

Fig. 2 | **Hypothetical model of an inverse correlation of the cellular peroxide tone and immune evasion.** Because the activity of prostaglandin-endoperoxide synthase 2 (PTGS2) is directly regulated through the cellular peroxide tone ([PL + PLOH]/[PLOOH]) via glutathione peroxidase 4 (GPX4), prostaglandin E_2 (PGE_2)-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 tone[72]. The peroxide tone refers to the steady-state level of cellular lipid hydroperoxides that is ultimately required for the activation of both LOXs and COXs[73]. 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 PGE_2 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 autoxidation catalysed by transition metals, ultimately leading to the formation of isoprostanes[76]. In this regard, it has been reported that ferroptosis-sensitive cells, and cells undergoing ferroptosis, are characterized by the massive release of oxidized lipid mediators[3], 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 eicosanoids[77]. 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 host[78]. 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), PGE_2, interleukin-6 (IL-6) and IL-1β, must also be considered[79]. 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 mediators[80]. TNF and IL-1β 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-1β[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 lethality[32]. Knockout of *Gpx4* in the myeloid lineage also stimulated intestinal tumorigenesis via increased genetic instability in intestinal epithelial cells[82]. 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 infections[83]. 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' signals[84]. 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 signals[85]. 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 depletion[3], whereas increasing GPX4 activity reduced pro-inflammatory lipid mediator production (5-HETE and LTB4) and inhibited pro-inflammatory features driven by NF-κB pathway activation in cells stimulated with TNF or IL-1β[86]. Notably, LTB4 is a well-established pro-inflammatory leukotriene with important roles in carcinogenesis[87].

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 roles[88]. 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

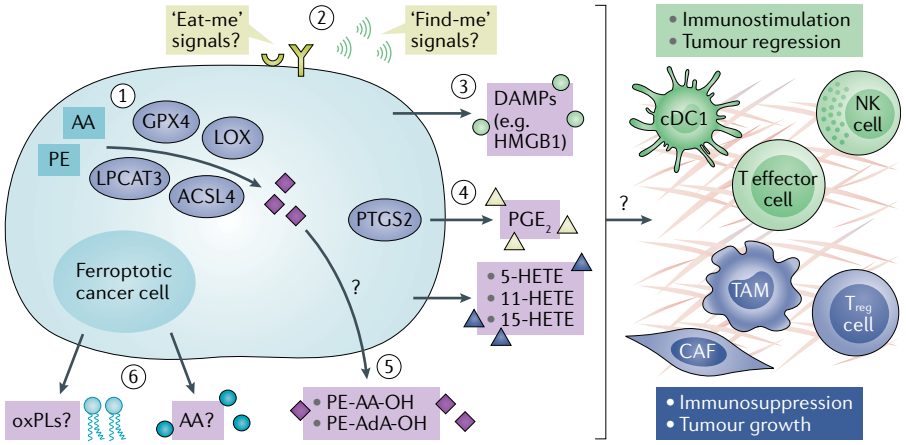

Fig. 3 | **Possible modulation of tumour immunity by ferroptotic cancer cells.** Sensitivity to ferroptosis is mediated by the activity of glutathione peroxidase 4 (GPX4), acyl-CoA synthetase long chain family member 4 (ACSL4), lyso-phosphatidylcholine acyltransferase 3 (LPCAT3) and lipoxygenases (LOXs) (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 phagocytes[85]. 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 death[96]. It is known that its release is required for immunogenicity of apoptotic cell death[105], 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 depletion[3] in addition to prostaglandin E_2 (PGE_2)[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 cells[90]. 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) cells[91].

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 signal[92]. 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 vivo[93].

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 cells[98]. 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 cells[96]. 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 manner[103], 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 manner[104]. 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 apoptosis[105]. 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 PGE_2, 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 PGE_2 (REF.[4]), it is conceivable that, if sufficient levels are achieved, a shift from antitumour to immunosuppressive responses might take place[106], leading to progressive tumour growth[107,108], although this requires experimental validation (FIG. 2). Interestingly, the release of PGE_2 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 PGE_2 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, PGE_2 production was sufficient to blunt conventional type 1 dendritic cell (cDC1)-dependent $CD8^+$ T cell-mediated immune control[107]. Further work demonstrated that PGE_2 inhibits the infiltration of cDC1s into the tumour site through the suppression of the chemokines CCL5 and XCL1 secreted by NK cells[108]. The action of PGE_2 and its downstream signalling were not studied in great detail, but early work provided evidence of a strong immunosuppressive effect of PGE_2 towards NK cells[109]. In addition to targeting NK cell activity, PGE_2 also hinders cDC1s directly by downregulating the chemokine receptors that promote recruitment into tumours. Therefore, PGE_2 impairs antitumour immunity by acting on at least two cell types of the innate immune system, NK cells and cDC1s.

In addition, PGE_2 also directly suppressed cytotoxic T cell action[63], underscoring its role as a major immunosuppressive mediator that interferes with multiple aspects of anticancer immunity. Recently, it was shown that, during chemotherapy cycles, PGE_2 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 PGE_2. In fact, chemotherapy targeting fast cycling cells would ultimately select these mesenchymal-like states, which are then poised to generate PGE_2, 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 activity[3]. 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 H_2O_2 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 RTK[124]. 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 H_2O_2 derived from mitochondria as well as from professional H_2O_2-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 H_2O_2 with PTPs, which afterward led to the concept that this oxidation might require a redox relay dependent on professional H_2O_2-removing enzymes, such as peroxiredoxins[125].

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 H_2O_2, 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 inihbitors[126]. 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 cells[127]. 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.

José Pedro Friedmann Angeli[1]*, *Dmitri V. Krysko*[2,3] *and Marcus Conrad*[4]**

[1]*Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany.*

[2]*Department of Human Structure and Repair, Ghent University and Cancer Research Institute Ghent (CRIG), Ghent, Belgium.*

[3]*Institute of Biology and Biomedicine, National Research Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russian Federation.*

[4]*Institute of Developmental Genetics, Helmholtz Zentrum München, Neuherberg, Germany.*

**e-mail: pedro.angeli@virchow.uni-wuerzburg.de; marcus.conrad@helmholtz-muenchen.de*

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1. Conrad, M., Angeli, J. P., Vandenabeele, P. & Stockwell, B. R. Regulated necrosis: disease relevance and therapeutic opportunities. *Nat. Rev. Drug Discov.* **15**, 348–366 (2016).
2. Dixon, S. J. et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **149**, 1060–1072 (2012).
3. Friedmann Angeli, J. P. et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat. Cell Biol.* **16**, 1180–1191 (2014).
4. Yang, W. S. et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell* **156**, 317–331 (2014).
5. Ingold, I. et al. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* **172**, 409–422 (2018).
6. Kagan, V. E. et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* **13**, 81–90 (2017).
7. Doll, S. et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* **13**, 91–98 (2017).
8. Dixon, S. J. et al. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem. Biol.* **10**, 1604–1609 (2015).
9. Yuan, H., Li, X., Zhang, X., Kang, R. & Tang, D. Identification of ACSL4 as a biomarker and contributor of ferroptosis. *Biochem. Biophys. Res. Commun.* **478**, 1338–1343 (2016).
10. Friedmann-Angeli, J. P., Miyamoto, S. & Schulze, A. Ferroptosis: the greasy side of cell death. *Chem. Res. Toxicol.* **32**, 362–369 (2019).
11. Chen, Y. et al. Quantitative profiling of protein carbonylations in ferroptosis by an aniline-derived probe. *J. Am. Chem. Soc.* **140**, 4712–4720 (2018).
12. Wang, L. et al. A pharmacological probe identifies cystathionine beta-synthase as a new negative regulator for ferroptosis. *Cell Death Dis.* **9**, 1005 (2018).
13. Lee, J. et al. The viability of primary hepatocytes is maintained under a low cysteine-glutathione redox state with a marked elevation in ophthalmic acid production. *Exp. Cell Res.* **361**, 178–191 (2017).
14. Hayano, M., Yang, W. S., Corn, C. K., Pagano, N. C. & Stockwell, B. R. Loss of cysteinyl-tRNA synthetase (CARS) induces the transsulfuration pathway and inhibits ferroptosis induced by cystine deprivation. *Cell Death Differ.* **23**, 270–278 (2016).
15. Gao, M., Monian, P., Quadri, N., Ramasamy, R. & Jiang, X. Glutaminolysis and transferrin regulate ferroptosis. *Mol. Cell* **59**, 298–308 (2015).
16. Gao, M. et al. Ferroptosis is an autophagic cell death process. *Cell Res.* **26**, 1021–1032 (2016).
17. Hou, W. et al. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* **12**, 1425–1428 (2016).
18. Torii, S. et al. An essential role for functional lysosomes in ferroptosis of cancer cells. *Biochem. J.* **473**, 769–777 (2016).
19. Conrad, M. et al. Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev.* **32**, 602–619 (2018).
20. Miess, H. et al. The glutathione redox system is essential to prevent ferroptosis caused by impaired lipid metabolism in clear cell renal cell carcinoma. *Oncogene* **37**, 5435–5450 (2018).
21. Viswanathan, V. S. et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* **547**, 453–457 (2017).
22. Jiang, L. et al. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* **520**, 57–62 (2015).
23. Tsoi, J. et al. Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. *Cancer Cell* **33**, 890–904 (2018).
24. Gentric, G. et al. PML-regulated mitochondrial metabolism enhances chemosensitivity in human ovarian cancers. *Cell Metab.* **29**, 156–173 (2019).
25. Brigelius-Flohe, R. & Maiorino, M. Glutathione peroxidases. *Biochim. Biophys. Acta* **1830**, 3289–3303 (2013).
26. Ursini, F., Maiorino, M., Valente, M., Ferri, L. & Gregolin, C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. *Biochim. Biophys. Acta* **710**, 197–211 (1982).
27. Yant, L. J. et al. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic. Biol. Med.* **34**, 496–502 (2003).
28. Liu, H., Schreiber, S. L. & Stockwell, B. R. Targeting dependency on the GPX4 lipid peroxide repair pathway for cancer therapy. *Biochemistry* **57**, 2059–2060 (2018).
29. Shah, R., Shchepinov, M. S. & Pratt, D. A. Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Cent. Sci.* **4**, 387–396 (2018).
30. Nomura, K., Imai, H., Koumura, T., Kobayashi, T. & Nakagawa, Y. Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome *c* from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia-induced apoptosis. *Biochem. J.* **351**, 183–193 (2000).
31. Canli, O. et al. Glutathione peroxidase 4 prevents necroptosis in mouse erythroid precursors. *Blood* **127**, 139–148 (2016).
32. Kang, R. et al. Lipid peroxidation drives gasdermin D-mediated pyroptosis in lethal polymicrobial sepsis. *Cell Host Microbe* **24**, 97–108 (2018).
33. Muller, T. et al. Necroptosis and ferroptosis are alternative cell death pathways that operate in acute kidney failure. *Cell. Mol. Life Sci.* **74**, 3631–3645 (2017).
34. Cheok, C. F., Verma, C. S., Baselga, J. & Lane, D. P. Translating p53 into the clinic. *Nat. Rev. Clin. Oncol.* **8**, 25–37 (2011).
35. Li, T. et al. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. *Cell* **149**, 1269–1283 (2012).
36. Wang, S. J. et al. Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. *Cell Rep.* **17**, 366–373 (2016).
37. Jennis, M. et al. An African-specific polymorphism in the TP53 gene impairs p53 tumor suppressor function in a mouse model. *Genes Dev.* **30**, 918–930 (2016).
38. Aydin, E., Johansson, J., Nazir, F. H., Hellstrand, K. & Martner, A. Role of NOX2-derived reactive oxygen species in NK cell-mediated control of murine melanoma metastasis. *Cancer Immunol. Res.* **5**, 804–811 (2017).
39. Moon, S. H. et al. p53 represses the mevalonate pathway to mediate tumor suppression. *Cell* **176**, 564–580 (2019).
40. Shimada, K. et al. Global survey of cell death mechanisms reveals metabolic regulation of ferroptosis. *Nat. Chem. Biol.* **12**, 497–503 (2016).
41. Garcia-Bermudez, J. et al. Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. *Nature* **567**, 118–122 (2019).
42. Turrell, F. K. et al. Lung tumors with distinct p53 mutations respond similarly to p53 targeted therapy but exhibit genotype-specific statin sensitivity. *Genes Dev.* **31**, 1339–1353 (2017).
43. Tarangelo, A. et al. p53 suppresses metabolic stress-induced ferroptosis in cancer cells. *Cell Rep.* **22**, 569–575 (2018).
44. Maddocks, O. D. et al. Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* **493**, 542–546 (2013).
45. Xie, Y. et al. The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. *Cell Rep.* **20**, 1692–1704 (2017).
46. Carbone, M. et al. BAP1 and cancer. *Nat. Rev. Cancer* **13**, 153–159 (2013).
47. Zhang, Y. et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat. Cell Biol.* **20**, 1181–1192 (2018).
48. Baughman, J. M. et al. NeuCode proteomics reveals Bap1 regulation of metabolism. *Cell Rep.* **16**, 583–595 (2016).
49. Faronato, M. et al. Increased expression of 5-lipoxygenase is common in clear cell renal cell carcinoma. *Histol. Histopathol.* **22**, 1109–1118 (2007).
50. Zou, Y. et al. A GPX4-dependent cancer cell state underlies the clear-cell morphology and confers sensitivity to ferroptosis. *Nat. Commun.* **10**, 1617 (2019).
51. Jiang, Y. et al. EGLN1/c-Myc induced lymphoid-specific helicase inhibits ferroptosis through lipid metabolic gene expression changes. *Theranostics* **7**, 3293–3305 (2017).
52. Briggs, K. J. et al. Paracrine induction of HIF by glutamate in breast cancer: EglN1 senses cysteine. *Cell* **166**, 126–139 (2016).
53. Timmerman, L. A. et al. Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. *Cancer Cell* **24**, 450–465 (2013).
54. Chen, X. et al. XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. *Nature* **508**, 103–107 (2014).
55. Accioly, M. T. et al. Lipid bodies are reservoirs of cyclooxygenase-2 and sites of prostaglandin-E2 synthesis in colon cancer cells. *Cancer Res.* **68**, 1732–1740 (2008).
56. Liu, L. et al. Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. *Cell* **160**, 177–190 (2015).
57. Behan, F. M. et al. Prioritization of cancer therapeutic targets using CRISPR–Cas9 screens. *Nature* **568**, 511–516 (2019).
58. Hangauer, M. J. et al. Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* **551**, 247–250 (2017).
59. Richard, G. et al. ZEB1-mediated melanoma cell plasticity enhances resistance to MAPK inhibitors. *EMBO Mol. Med.* **8**, 1143–1161 (2016).
60. Talebi, A. et al. Sustained SREBP-1-dependent lipogenesis as a key mediator of resistance to BRAF-targeted therapy. *Nat. Commun.* **9**, 2500 (2018).
61. Krebs, A. M. et al. The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nat. Cell Biol.* **19**, 518–529 (2017).
62. Gubelmann, C. et al. Identification of the transcription factor ZEB1 as a central component of the adipogenic gene regulatory network. *eLife* **3**, e03346 (2014).
63. Wang, D. & DuBois, R. N. Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis* **36**, 1085–1093 (2015).
64. Di Minin, G. et al. Mutant p53 reprograms TNF signaling in cancer cells through interaction with the tumor suppressor DAB2IP. *Mol. Cell* **56**, 617–629 (2014).
65. Wang, S. S. et al. Bap1 is essential for kidney function and cooperates with Vhl in renal tumorigenesis. *Proc. Natl Acad. Sci. USA* **111**, 16538–16543 (2014).
66. Kalluri, R. & Weinberg, R. A. The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* **119**, 1420–1428 (2009).
67. Wei, J. & Gronert, K. Eicosanoid and specialized proresolving mediator regulation of lymphoid cells. *Trends Biochem. Sci.* **44**, 214–225 (2018).
68. Chen, C. J., Huang, H. S. & Chang, W. C. Inhibition of arachidonate metabolism in human epidermoid carcinoma a431 cells overexpressing phospholipid hydroperoxide glutathione peroxidase. *J. Biomed. Sci.* **9**, 453–459 (2002).
69. Chen, C. J., Huang, H. S., Lin, S. B. & Chang, W. C. Regulation of cyclooxygenase and 12-lipoxygenase catalysis by phospholipid hydroperoxide glutathione peroxidase in A431 cells. *Prostaglandins Leukot. Essent. Fatty Acids* **62**, 261–268 (2000).
70. Huang, H. S., Chen, C. J., Suzuki, H., Yamamoto, S. & Chang, W. C. Inhibitory effect of phospholipid hydroperoxide glutathione peroxidase on the activity of lipoxygenases and cyclooxygenases. *Prostaglandins Other Lipid Mediat.* **58**, 65–75 (1999).
71. Chen, C. J., Huang, H. S. & Chang, W. C. Depletion of phospholipid hydroperoxide glutathione peroxidase up-regulates arachidonate metabolism by 12S-lipoxygenase and cyclooxygenase 1 in human epidermoid carcinoma A431 cells. *FASEB J.* **17**, 1694–1696 (2003).

72. Araujo, A. C., Wheelock, C. E. & Haeggstrom, J. Z. The eicosanoids, redox-regulated lipid mediators in immunometabolic disorders. *Antioxid. Redox Signal.* **29**, 275–296 (2018).
73. Haeggstrom, J. Z. & Funk, C. D. Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. *Chem. Rev.* **111**, 5866–5898 (2011).
74. Seiler, A. et al. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* **8**, 237–248 (2008).
75. Loscalzo, J. Membrane redox state and apoptosis: death by peroxide. *Cell Metab.* **8**, 182–183 (2008).
76. Milne, G. L., Dai, Q. & Roberts, L. J. 2nd. The isoprostanes—25 years later. *Biochim. Biophys. Acta* **1851**, 433–445 (2015).
77. Folco, G. & Murphy, R. C. Eicosanoid transcellular biosynthesis: from cell-cell interactions to in vivo tissue responses. *Pharmacol. Rev.* **58**, 375–388 (2006).
78. Dar, H. H. et al. *Pseudomonas aeruginosa* utilizes host polyunsaturated phosphatidylethanolamines to trigger theft-ferroptosis in bronchial epithelium. *J. Clin. Invest.* **128**, 4639–4653 (2018).
79. Kim, S. et al. Circulating levels of inflammatory cytokines and risk of colorectal adenomas. *Cancer Res.* **68**, 323–328 (2008).
80. Latchoumycandane, C., Marathe, G. K., Zhang, R. & McIntyre, T. M. Oxidatively truncated phospholipids are required agents of tumor necrosis factor alpha (TNFalpha)-induced apoptosis. *J. Biol. Chem.* **287**, 17693–17705 (2012).
81. Katunga, L. A. et al. Obesity in a model of gpx4 haploinsufficiency uncovers a causal role for lipid-derived aldehydes in human metabolic disease and cardiomyopathy. *Mol. Metab.* **4**, 493–506 (2015).
82. Canli, O. et al. Myeloid cell-derived reactive oxygen species induce epithelial mutagenesis. *Cancer Cell* **32**, 869–883 (2017).
83. Matsushita, M. et al. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. *J. Exp. Med.* **212**, 555–568 (2015).
84. Elliott, M. R. & Ravichandran, K. S. The dynamics of apoptotic cell clearance. *Dev. Cell* **38**, 147–160 (2016).
85. Kloditz, K. & Fadeel, B. Three cell deaths and a funeral: macrophage clearance of cells undergoing distinct modes of cell death. *Cell Death Discov.* **5**, 65 (2019).
86. Li, C. et al. Novel allosteric activators for ferroptosis regulator glutathione peroxidase 4. *J. Med. Chem.* **62**, 266–275 (2018).
87. Wang, D. & Dubois, R. N. Eicosanoids and cancer. *Nat. Rev. Cancer* **10**, 181–193 (2010).
88. Morgan, A. H. et al. Phosphatidylethanolamine-esterified eicosanoids in the mouse: tissue localization and inflammation-dependent formation in Th-2 disease. *J. Biol. Chem.* **284**, 21185–21191 (2009).
89. D'Herde, K. & Krysko, D. V. Ferroptosis: oxidized PEs trigger death. *Nat. Chem. Biol.* **13**, 4–5 (2017).
90. Lauber, K. et al. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell* **113**, 717–730 (2003).
91. Rothe, T. et al. 12/15-Lipoxygenase-mediated enzymatic lipid oxidation regulates DC maturation and function. *J. Clin. Invest.* **125**, 1944–1954 (2015).
92. Tyurin, V. A. et al. Oxidatively modified phosphatidylserines on the surface of apoptotic cells are essential phagocytic 'eat-me' signals: cleavage and inhibition of phagocytosis by Lp-PLA2. *Cell Death Differ.* **21**, 825–835 (2014).
93. Uderhardt, S. et al. 12/15-lipoxygenase orchestrates the clearance of apoptotic cells and maintains immunologic tolerance. *Immunity* **36**, 834–846 (2012).
94. Veglia, F. et al. Lipid bodies containing oxidatively truncated lipids block antigen cross-presentation by dendritic cells in cancer. *Nat. Commun.* **8**, 2122 (2017).
95. Cao, W. et al. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. *J. Immunol.* **192**, 2920–2931 (2014).
96. Wen, Q., Liu, J., Kang, R., Zhou, B. & Tang, D. The release and activity of HMGB1 in ferroptosis. *Biochem. Biophys. Res. Commun.* **510**, 278–283 (2019).
97. Yu, Y. et al. The ferroptosis inducer erastin enhances sensitivity of acute myeloid leukemia cells to chemotherapeutic agents. *Mol. Cell Oncol.* **2**, e1054549 (2015).
98. Yamazaki, T. et al. Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists. *Cell Death Differ.* **21**, 69–78 (2014).
99. Aaes, T. L. et al. Vaccination with necroptotic cancer cells induces efficient anti-tumor immunity. *Cell Rep.* **15**, 274–287 (2016).
100. Yatim, N. et al. RIPK1 and NF-kappaB signaling in dying cells determines cross-priming of $CD8^+$ T cells. *Science* **350**, 328–334 (2015).
101. Linkermann, A. et al. Synchronized renal tubular cell death involves ferroptosis. *Proc. Natl Acad. Sci. USA* **111**, 16836–16841 (2014).
102. Hassannia, B. et al. Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. *J. Clin. Invest.* **128**, 3341–3355 (2018).
103. Li, W. et al. Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. *J. Clin. Invest.* **130**, 126428 (2019).
104. Krysko, D. V. et al. TLR-2 and TLR-9 are sensors of apoptosis in a mouse model of doxorubicin-induced acute inflammation. *Cell Death Differ.* **18**, 1316–1325 (2011).
105. Apetoh, L. et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
106. Wang, D. & DuBois, R. N. The role of prostaglandin E(2) in tumor-associated immunosuppression. *Trends Mol. Med.* **22**, 1–3 (2016).
107. Zelenay, S. et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* **162**, 1257–1270 (2015).
108. Bottcher, J. P. et al. NK Cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* **172**, 1022–1037 (2018).
109. Kalinski, P. Regulation of immune responses by prostaglandin E2. *J. Immunol.* **188**, 21–28 (2012).
110. Kurtova, A. V. et al. Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nature* **517**, 209–213 (2015).
111. Bluml, S. et al. Oxidized phospholipids negatively regulate dendritic cell maturation induced by TLRs and CD40. *J. Immunol.* **175**, 501–508 (2005).
112. Dixon, S. J. et al. Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *eLife* **3**, e02523 (2014).
113. Yoshikawa, M. et al. xCT inhibition depletes CD44v-expressing tumor cells that are resistant to EGFR-targeted therapy in head and neck squamous cell carcinoma. *Cancer Res.* **73**, 1855–1866 (2013).
114. Zhang, Y. et al. Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. *Cell Chem. Biol.* https://doi.org/10.1016/j.chembiol.2019.01.008 (2019).
115. Chen, M. S. et al. CHAC1 degradation of glutathione enhances cystine-starvation-induced necroptosis and ferroptosis in human triple negative breast cancer cells via the GCN2-eIF2alpha-ATF4 pathway. *Oncotarget* **8**, 114588–114602 (2017).
116. Mai, T. T. et al. Salinomycin kills cancer stem cells by sequestering iron in lysosomes. *Nat. Chem.* **9**, 1025–1033 (2017).
117. Hao, S. et al. Cysteine dioxygenase 1 mediates erastin-induced ferroptosis in human gastric cancer cells. *Neoplasia* **19**, 1022–1032 (2017).
118. Guo, J. et al. Ferroptosis: a novel anti-tumor action for cisplatin. *Cancer Res. Treat.* **50**, 445–460 (2018).
119. Fang, X. et al. Ferroptosis as a target for protection against cardiomyopathy. *Proc. Natl Acad. Sci. USA* **116**, 2672–2680 (2019).
120. Yao, X. et al. Multifunctional nanoplatform for photoacoustic imaging-guided combined therapy enhanced by CO induced ferroptosis. *Biomaterials* **197**, 268–283 (2019).
121. Kim, S. E. et al. Ultrasmall nanoparticles induce ferroptosis in nutrient-deprived cancer cells and suppress tumour growth. *Nat. Nanotechnol.* **11**, 977–985 (2016).
122. Szwed, M. et al. Small variations in nanoparticle structure dictate differential cellular stress responses and mode of cell death. *Nanotoxicology* https://doi.org/10.1080/17435390.2019.1576238 (2019).
123. Wang, S. et al. Arginine-rich manganese silicate nanobubbles as a ferroptosis-inducing agent for tumor-targeted theranostics. *ACS Nano.* **12**, 12380–12392 (2018).
124. Ostman, A., Frijhoff, J., Sandin, A. & Bohmer, F. D. Regulation of protein tyrosine phosphatases by reversible oxidation. *J. Biochem.* **150**, 345–356 (2011).
125. Stocker, S., Maurer, M., Ruppert, T. & Dick, T. P. A role for 2-Cys peroxiredoxins in facilitating cytosolic protein thiol oxidation. *Nat. Chem. Biol.* **14**, 148–155 (2018).
126. Conrad, M. et al. 12/15-lipoxygenase-derived lipid peroxides control receptor tyrosine kinase signaling through oxidation of protein tyrosine phosphatases. *Proc. Natl Acad. Sci. USA* **107**, 15774–15779 (2010).
127. Wang, H. P., Schafer, F. Q., Goswami, P. C., Oberley, L. W. & Buettner, G. R. Phospholipid hydroperoxide glutathione peroxidase induces a delay in G1 of the cell cycle. *Free Radic. Res.* **37**, 621–630 (2003).
128. Martin-Sanchez, D. et al. TWEAK and RIPK1 mediate a second wave of cell death during AKI. *Proc. Natl Acad. Sci. USA* **115**, 4182–4187 (2018).

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