Non-neuroendocrine differentiation generates a ferroptosis-prone lipidome in small cell lung cancer (SCLC)

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ABSTRACT

Our recent study revealed that non-neuroendocrine small cell lung cancer (SCLC) is sensitive to the induction of ferroptosis due to upregulation of ether lipid synthesis. While neuroendocrine SCLC is ferroptosis resistant, it acquires addiction to the thioredoxin pathway. Combined redox pathway targeting therefore achieves efficient anti-tumor activity in heterogeneous SCLC.

Cell death is mainly caused by two different types of cell death, accidental cell death (ACD) and programmed cell death (PCD), an important regulated mechanism controlling essential physiological processes such as development, immunity and tissue homeostasis. PCD can be triggered by intracellular or extracellular perturbations overcoming stress response mechanisms in cells which in turn activate a “programmed” molecular cascade ultimately resulting in cell death. The most in-depth researched PCD, to date, is apoptosis which can be induced by intracellular triggers such as DNA damage or stress signals resulting in an activation of pro-apoptotic over anti-apoptotic factors leading to mitochondria outer membrane permeabilization (MOMP). Cytochrome C released from mitochondria then activates the caspase (CASP) cascade consisting of initiator CASP8/9/10 and effector CASP3/6/7 preventing uncontrolled membrane rupture and cell content release. Similarly, extrinsic apoptosis is induced upon binding of death ligands belonging to the tumor necrosis factor (TNF) superfamily to their cognate receptors on the cell surface resulting in either direct recruitment of Fas-associated protein with death domain (FADD) to membrane complexes or intracellular secondary complexes followed by activation of CASP cascade. Contrarily, necrotic PCDs such as necroptosis result in membrane rupture accompanied by a release of cellular content. Upon extra- or intracellular stimulation, the necroptotic machinery depends on the sequential activation of receptor interacting serine/threonine kinase 1 (RIPK1) activating RIPK3 and mixed lineage kinase domain like pseudokinase (MLKL) forming the necrosome triggering membrane permeabilization.

These tightly regulated processes are often inactivated in prevalent human pathologies such as cancer. Fortunately, PCD can often be re-instated through genetic or pharmacological interventions enabling therapy options for these cancers. Moreover, the recent advent of immunotherapy has exploited the fact that also immune effector cells can be re-activated to effectively trigger PCD in tumor cells. Herein, binding of death ligands expressed by immune effector cells to their cognate receptor on tumor cells induces extrinsic apoptosis and/or necroptosis. Interestingly, ferroptosis – another type of regulated necrosis - was recently described to be amplified by interferon gamma (IFNγ) released from CD8+ T cell activated by immunotherapy\(^1\).

So far, execution of ferroptosis has been described as a collapse of the cellular antioxidant defense machinery rather than as an agonist-driven molecular cascade actively inducing cell death. An important hallmark of ferroptosis is the characteristic accumulation of peroxided membrane lipids, allowing for membrane pore formation and destabilization of the lipid bilayer\(^2\). Within the plasma membrane, polyunsaturated fatty acids (PUFAs) within phospholipids harboring methylene bridges with highly reactive hydrogen atoms are especially sensitive to lipid peroxidation which are by orders of magnitude less peroxidized during other types of PCD\(^3\). Furthermore, ether-linked phospholipids synthesized in peroxisomes were recently identified to substantially contribute to the lipid peroxide pool and thereby drive ferroptosis susceptibility\(^4\).

Lipid peroxidation can take place spontaneously in the presence of free redox active divalent iron (Fe\(^{2+}\)) to promote a Fenton reaction generating hydroxyl radicals (HO-) or Fe\(^{3+}\) serves as a cofactor for lipoxygenase (LOX) to catalyze peroxidation enzymatically.

In order to protect cells from spontaneous or enzymatically catalyzed lipid peroxidation, glutathione peroxidase 4 (GPX4) constitutively hydrolyzes lipid hydroperoxides (L-OOH) in a glutathione (GSH)-dependent manner and thereby prevents ferroptosis\(^5\). Recently, ferroptosis suppressor protein 1 (FSP1)
was shown to be recruited to the plasma membrane where it acts as an oxidoreductase reducing ubiquinone (=Coenzyme Q10) to the lipophilic radical scavenger ubiquinol preventing lipid peroxidation in the absence of GPX4.\textsuperscript{6,7}

Amongst lung cancer entities, small cell lung cancer (SCLC) is the most aggressive form, defined by highly proliferative, small cells mostly of neuroendocrine origin with a high tumor mutational burden (TMB) closely associated with tobacco smoking. SCLC often presents with heterogeneous tumors consisting of neuroendocrine (NE) and non-neuroendocrine (non-NE) cells, a fact complicating therapy. Despite initial response of most SCLC patients to chemotherapy, they almost invariably develop relapse.

In our recent study\textsuperscript{8}, we surprisingly identified that in treatment-naïve SCLC patient tumor tissue expression of many PCD pathway components, including those driving apoptosis and necroptosis, was already lost at diagnosis. These data strongly suggest that SCLC likely undergoes stringent selection against PCD pathways prior to therapy. As SCLC is known to present with high TMB, this prior selection pressure may stem from immune effector cells trying to kill tumor cells with high TMB. Interestingly, neuroendocrine status of SCLC has recently been linked to immune gene signature expression\textsuperscript{9}. NE cells repress expression of immune signature genes especially major histocompatibility complex (MHC) I, while MHC I proteins were described to be re-expressed on non-NE chemoresistant relapsed cells in some cases.

Despite this broad inactivation of PCD, we identified that non-NE mouse and human SCLC cells show a strong response to ferroptosis inducing agents, while NE SCLC cells are instead highly vulnerable to thioredoxin (TRX) pathway inhibition. Importantly, we found that non-NE differentiation, an SCLC subtype associated with chemotherapy resistance upon relapse, renders SCLC exquisitely ferroptosis sensitive by upregulating ether-linked PUFA synthesis resulting in a ferroptosis-prone lipidome (Figure 1).

Metabolic remodeling is one of the hallmarks of cancer and changes in lipid metabolism have been described to fuel cancer progression and proliferation. Interestingly, Acyl-CoA synthetase long-chain family member 4 (ACSL4), important for PUFA phospholipid synthesis, has been reported to increase chemoresistance in breast cancer by regulating ATP binding cassette (ABC) transporter expression\textsuperscript{10} hinting at the possibility that acquired metabolic changes in NE to non-NE plasticity

![Figure 1. Phenotypic heterogeneity of small cell lung cancer (SCLC): Heterogeneous SCLC tumors contain non-neuroendocrine (non-NE) and neuroendocrine (NE) cells. Non-NE cell membranes are characterized by a high arachidonic acid (AA) and adrenic acid (AdA) phospholipid and ether-linked polyunsaturated fatty acid (PUFA) phospholipid content prone to be peroxidized during ferroptosis. Acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are crucial for the synthesis of AA and AdA containing phospholipids and alkylglycerone phosphate synthase (AGPS) and 1-acylglycerol-3-phosphate O-acyltransferase 3 (AGPAT3) catalyze ether phospholipid synthesis from Acyl-Coenzyme A (Acyl-CoA) and are highly expressed in non-NE SCLC cells. Peroxidation of membrane lipids is counteracted by glutathione peroxidase 4 (GPX4) reducing lipid peroxides (L-OH) to their respective lipid alcohol (L-OH) while oxidizing glutathione (GSH) to glutathione disulfide (GSSG) thereby preventing ferroptosis. NE SCLC cells present with a lipidome less prone to ferroptosis-induced lipid peroxidation and are highly dependent on the thioredoxin (TRX) redox pathway. Here, reactive oxygen (ROS) species such as hydrogen peroxide (H\(_2\)O\(_2\)) as well as lipid peroxides are reduced respectively by peroxiredoxins (PRDX) using TRX as a redox equivalent, which is restored by thioredoxin reductases (TRXR). Inhibition of the thioredoxin pathways results in ROS-dependent cell death in NE SCLC. The scheme was drawn using fully licensed Biorender.com.](#)
may further fuel chemotherapy resistance of non-NE SCLC. Yet, the exact mechanism by which increased lipid metabolism might support chemoresistance in non-NE SCLC remains to be further examined. Based on our finding that non-NE SCLC is specifically ferroptosis sensitive due to elevated ether lipid synthesis, ferroptosis induction could serve as alternative treatment strategy specifically targeting non-NE chemoresistant tumors upon relapse.

Of note, our study also showed that NE SCLC did not present with elevated ether lipid synthesis and was resistant to ferroptosis. Instead, we identified that NE cells rely on the TRX pathway for antioxidant defense and Auranofin, a thioredoxin reductase (TRXR) inhibitor, which is already clinically approved for the treatment of rheumatoid arthritis, was highly and selectively active against NE SCLC cells. Consequently, we also identified a novel Achilles heel of the NE SCLC subtype. Importantly, the combined therapy of ferroptosis induction and targeting the TRX pathway allows to target heterogenous SCLC tumors and subtype plasticity oppressing escape mechanisms.

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No potential conflicts of interest were disclosed.

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