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DUBbing ferroptosis in cancer cells

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Significance

Ferroptosis, a form of iron-dependent, non-apoptotic cell death that is induced by excessive lipid peroxidation, has been recently identified as a new tumor suppression mechanism. In this issue of *Cancer Research*, Liu and colleagues demonstrate that the deubiquitinase (DUB) OTUB1 is frequently overexpressed in human cancers, and functions to "dub" (trim) the ferroptosis process in cancer cells and promotes tumor development by stabilizing the cystine transporter SLC7A11. This study not only reveals a hitherto unappreciated regulatory mechanism of ferroptosis but also identifies potential therapeutic targets for cancer treatment.

Ferroptosis was discovered in 2012 by Stockwell and colleagues as a form of iron-dependent regulated cell death that is caused by a lethal accumulation of lipid peroxidation (1). Accumulating evidence indicates that ferroptosis is mainly induced by peroxidation of phospholipids containing polyunsaturated fatty acids (PUFAs, fatty acids that contain more than one double bond). Incorporation of PUFAs into cellular membranes is beneficial to multicellular organisms because PUFAs increases membrane fluidity and cell-signaling capabilities. However, these benefits come at a significant cost: cellular membranes with more PUFAs are more susceptible to lipid peroxidation, and excessive lipid peroxidation in cellular membranes leads to ferroptosis (2). Cells have evolved elegant defense mechanisms against ferroptosis to eliminate these harmful lipid peroxides. These mainly involve an enzyme called glutathione peroxidase 4 (GPX4), which utilizes glutathione as a co-factor to convert lipid peroxides to lipid alcohols, thus preventing cells from accumulating too much lipid peroxidation (2). Correspondingly, inactivating GPX4 through pharmacologic or genetic means induces ferroptosis both in vitro and in vivo (3). Glutathione is a tripeptide that is made up of glutamate, glycine, and cysteine, which is the rate-limiting precursor. Although some cells can synthesize cysteine through the transsulfuration pathway, many cancer cells rely on transport of extracellular cystine, the oxidized dimer form of cysteine, as their source of cysteine (4). Extracellular cystine is transported into cells mainly through an amino acid transporter called SLC7A11 (also known as xCT), which imports cystine and exports glutamate at a 1:1 ratio (5). Once imported into cells, intracellular cystine is reduced to cysteine, which is used for subsequent glutathione biosynthesis. Correspondingly,

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removing cystine in cell culture medium or blocking SLC7A11-mediated cystine transport by erastin induces ferroptosis in many cell lines (4).

Most known forms of regulated cell death have normal physiological functions, but when dysregulated, play a role in disease processes. One example is apoptosis, which serves as a "sculptor" to shape our hands and feet during embryonic development by eliminating cells between individual digits (digit individualization). Cancer cells have evolved various mechanisms to avoid apoptotic cell death. Dysregulation of ferroptosis has been associated with several pathologic conditions and diseases including kidney degeneration, ischemia-reperfusion injury, stroke, neurodegeneration, and cancer (2). The normal physiological function of ferroptosis still remains largely unknown. Arguably, the best evidence so far to support a physiological function of ferroptosis relates to its role in tumor suppression. Several tumor suppressors, including p53, BAP1, and KEAP1, have been identified as positive regulators of ferroptosis. Inactivation of these tumor suppressors has been shown to lead to ferroptosis resistance, which at least partly contributes to tumor development (2, 6, 7). Conceivably, ferroptosis may function as a tumor suppression mechanism in certain organs to eliminate precancerous cells and to prevent tumor development. Consequently, strong selection pressure probably exists to inactivate ferroptosis in these organs to promote tumor formation.

A central node connecting ferroptosis to tumor suppression is SLC7A11. The three tumor suppressors mentioned above have been shown to promote ferroptosis, at least in part, by suppressing *SLC7A11* expression. p53 binds to the *SLC7A11* promoter, directly suppressing its transcription (6); the nuclear deubiquitinase (DUB) BAP1 represses *SLC7A11* transcription by removing histone 2A ubiquitination from the *SLC7A11* promoter (7); and KEAP1 represses *SLC7A11* transcription through degrading NRF2, a master transcription factor of antioxidant response and regulator of *SLC7A11* (8). Consistent with these findings, SLC7A11 is often overexpressed in human cancers (4).

While most current studies including those described above have focused on the transcriptional regulation of SLC7A11 in cancer cells, whether and how SLC7A11 can be regulated at the post-transcriptional or post-translational levels is not well understood. In this issue of *Cancer Research*, Liu and colleagues identified a post-translational mechanism to control ferroptosis in cancer cells, which involves regulation of SLC7A11 protein stability by a DUB (9). The authors first set out to identify potential SLC7A11 regulators through affinity purification followed by mass spectrometry and identified not only known SLC7A11-interacting proteins such as SLC3A2, but also OTUB1, a DUB of the ovarian tumor (OTU) family, as a novel binding partner of SLC7A11. Consistent with the role of a DUB in regulating protein stability, *OTUB1* overexpression resulted in reduced SLC7A11 ubiquitination and increased SLC7A11 protein half-life and steady protein levels; conversely, *OTUB1* deletion in a variety of cancer cell lines resulted in a significant decrease in SLC7A11 protein levels. *OTUB1* deficiency inhibited cystine uptake and markedly sensitized cancer cells to ferroptosis induced by various ferroptosis inducers including *tert*-butyl hydroperoxide, erastin, and cystine deprivation. Restoring SLC7A11 in *OTUB1*-deficient cells reversed the increased ferroptosis sensitivity caused by *OTUB1* deletion, suggesting that *OTUB1* deletion promotes ferroptosis mainly through SLC7A11.

Analysis of *OTUB1* expression in human tumors and corresponding normal tissues revealed significantly increased expression of *OTUB1* in several forms of human cancer, most notably bladder cancer. Indeed, depleting *OTUB1* in bladder cancer cells drastically inhibited xenograft tumor development; notably, restoring SLC7A11 in *OTUB1*-deficient xenograft tumors (which exhibited decreased SLC7A11 protein levels) also largely restored tumor development. *OTUB1*-deficient xenograft tumors exhibited markedly increased expression of *PTGS2*, a ferroptosis marker (2), and this increase again was significantly mitigated by SLC7A11 restoration in *OTUB1*-deficient tumors, suggesting that OTUB1 promotes bladder tumor development through stabilizing SLC7A11 and inhibiting ferroptosis. It should be noted that *OTUB1* knockout (KO) cancer cells do not exhibit obvious ferroptosis under normal culture conditions, undergoing extensive ferroptosis only when treated with ferroptosis inducers (such as erastin) or cultured in cystine-free medium, whereas *OTUB1* KO tumors exhibit high expression of the ferroptosis marker and likely high ferroptosis in vivo in the absence of any ferroptosis inducer treatment. This is most likely because cultured cells are provided with sufficient cystine from the culture medium, whereas poor tumor vasculature often results in limited supply of nutrients including cystine in tumors, thus rendering tumor cells within the tumor mass more susceptible to ferroptosis than those cultured in vitro.

CD44, an adhesion molecule that is often highly expressed in cancer stem-like cells, promotes tumor development through interacting with and stabilizing SLC7A11 (10), although the underlying mechanism remains unclear. In this study, Liu and colleagues showed that SLC7A11, OTUB1, and CD44 form a tri-protein complex and that CD44 promotes both SLC7A11-OTUB1 interaction and SLC7A11 protein stability. *CD44* overexpression significantly increased SLC7A11 protein levels and inhibited erastin-induced ferroptosis, but these effects were abrogated in *OTUB1* KO cells, suggesting that CD44 regulates SLC7A11 protein stability and ferroptosis through OTUB1 and likely by promoting SLC7A11-OTUB1 interaction.

Collectively, these findings identified the first DUB to control SLC7A11 protein stability and a previously unrecognized regulatory mechanism to inactivate ferroptosis in cancer cells. This intriguing study has several important implications. It is conceivable that, similar to other important cellular processes, ferroptosis is tightly controlled by intricate regulatory mechanisms, although the exact mechanisms involved in ferroptosis regulation still remain poorly understood. This study will motivate further studies to identify other regulatory mechanisms (such as ubiquitination, phosphorylation, or other post-translational modifications) involved in ferroptosis pathways. This study further validates the concept that ferroptosis is an important tumor suppression mechanism. Cancer cells are likely selected to inactivate ferroptosis through multiple mechanisms, such as by de-repressing *SLC7A11* expression through inactivating *p53* or *BAP1* (6, 7) or by stabilizing SLC7A11 protein through upregulating OTUB1 or CD44 (9). Future studies will undoubtedly identify additional mechanisms linking ferroptosis to tumor biology. Finally, this study also suggests that OTUB1 and CD44 may represent novel therapeutic targets for inducing ferroptosis in cancer therapy.

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