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findings, mice lacking a functional A-type lamin gene (*Lmna*) develop an EDMD-like syndrome soon after birth and die within 6 weeks of cardiovascular disease. A recent publication by Chen et al. (2012) reports the remarkable finding that down-regulation of Sun1 expression in *Lmna*-deficient mice significantly extended their life span along with amelioration of cardiac pathology. Thus, LINC complexes could represent viable therapeutic targets for the treatment of certain types of striated muscle disease and perhaps other laminopathies. The insights that Sosa et al. now provide into the molecular details of LINC complex assembly suggest strategies for the design of drugs that could interfere with SUN-KASH interactions. This convergence of structural biology and mouse genetics may provide the foundation for novel interventions in muscular dystrophy and heart disease.

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Ironing Out Cell Death Mechanisms

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Historically, key cellular regulators of diverse physiological processes have been uncovered by studying the mechanisms by which chemical entities produce interesting biological phenotypes. In this issue, Dixon et al. interrogate compounds that selectively kill oncogene-expressing cells, providing support for the existence of an iron-requiring, regulated form of cell death, ferroptosis.

Various forms of regulated cell death have been identified, including apoptosis, necroptosis, paraptosis, parthanoptosis, pyroptosis, and autophagic cell death. In this issue, Stockwell and colleagues propose the existence of ferroptosis, an iron-dependent form of cell death (Dixon et al., 2012). Ferroptosis requires iron-dependent production of reactive oxygen species (ROS), involves nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases and lipid peroxidation, and is associated morphologically with the presence of shrunken, electron-dense mitochondria (Figure 1). Distinguishing it from many other forms of regulated cell death, ferroptosis does not require caspases (mediators of apoptosis and pyroptosis), ATP depletion or mitochondrial ROS generation (mediators of necroptosis), Bax/Bak (essential mediators of mitochondrial outer membrane permeabilization, MOMP), or elevations in intracellular Ca^{2+}.

Ferroptosis was uncovered while seeking an understanding of the mechanism underlying the activity of erastin, a small molecule that selectively kills cells expressing oncogenic mutants of RAS (Dolma et al., 2003). This quinazoline compound binds certain voltage-dependent anion channels (VDACs) on the mitochondrial outer membrane. Moreover, short hairpin RNA (shRNA) reagents targeting VDAC2 and VDAC3 rescue oncogenic RAS-expressing tumor cells from erastin (Yagoda et al., 2007; Yang and Stockwell, 2008). In the current paper, Dixon et al. (2012) show that erastin binds the subunit of cell surface amino acid transporters that import cystine, which then presumably causes reductions in glutathione, sensitizing cells to ROS. Evidence that cystine transport is critical to the mechanism of erastin includes data from experiments that either pharmacologically bypass erastin-induced cysteine transport block or that block cysteine transport with other compounds such as sulfasalazine and glutamate. The authors also sensitized tumor cells to erastin by shRNA-mediated knockdown of a subunit (SLCA11) of amino acid transporters

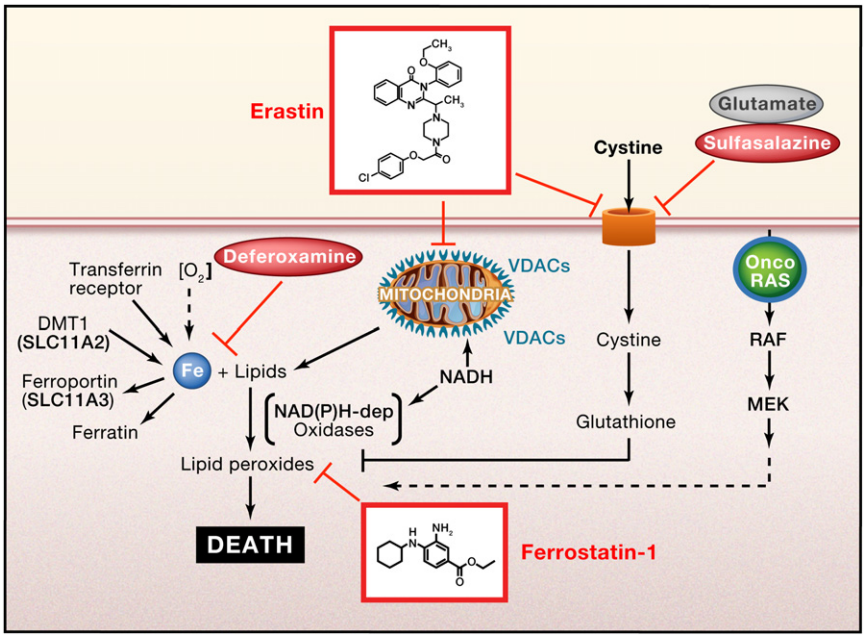

Figure 1. Ferroptosis—Cell Death by Iron-Dependent, Oxidative Injury

Ferroptosis is proposed to result from overwhelming iron-dependent oxidative injury, probably involving lipid peroxidation. The cellular factors responsible for generating ROS include NADPH-dependent oxidases. Some of the pathways impacting cellular sensitivity to ferroptosis are depicted, including regulators of iron homeostasis, RAS pathway induction of oxidase gene expression, and cystine transport impacting glutathione synthesis. Erastin modulates both cystine transport and VDAC-mediated NADH transport. Other chemical modulators are highlighted in red, including the newly reported ferrostatin.

and blunted erastin-mediated cytotoxicity by overexpressing the transporter subunit. Together, these data support the conclusion that erastin inhibits cysteine import. However, to definitively determine whether cystine transport is the critical cellular target of erastin that explains its cytotoxic activity against RAS-expressing cells, a thorough structure-activity relationship (SAR) analysis is required using various erastin analogs, including existing active versus inactive enantiomers.

Considering that previous studies have illustrated that chemical oxidants induce cytotoxicity of RAS-transformed cells (Trachootham et al., 2006), the novelty of the proposed new cell death mechanism is not that cystine-dependent glutathione production is altered by erastin but that this form of cell death requires iron. Screening a mitochondria-focused shRNA library revealed iron regulatory protein 2 (IRB2) as a target that promotes erastin-induced killing. Conversely, knocking down expression of the endogenous IRB2 antagonist FBXL5 sensitized cells to erastin. IRB2 is an RNA-binding protein that controls the translation of a group of mRNAs involved in iron homeostasis, decreasing iron uptake by reducing transferrin receptor expression and sequestering free Fe(II) by inducing ferratin expression. These and other experiments, such as reducing erastin-induced cytotoxicity with shRNA directed toward transferrin receptors, suggest that the pool of labile iron controls sensitivity to erastin. The authors also showed a role for NADPH-dependent oxidases that oncogenic RAS upregulates, including NADPH oxidase (NOX1), which interacts with protein complexes that contain Fe/heme-binding proteins.

Roles for iron in cell death have been recognized for many years in brain injury and neurodegeneration (Aracena et al., 2006). For example, elevated levels of iron are found in the substantia nigra of patients with Parkinson's disease and have also been reported in Alzheimer's brains. Iron chelation shows neuroprotective effects in models of neurotoxicity induced by 6-hydroxydopamine and MPP^+ (dopaminergic neurons) and also by amyloid-β peptide. Iron chelators have also shown neuroprotective activity in some animal models of neurotrauma. Moreover, *N*-methyl-D-aspartate (NMDA) receptors, mediators of L-glutamate-induced neuronal cell death, are reported to induce Fe(II) import into neurons. Further, L-glutamate was reported previously to impact cystine transport in neurons (Murphy et al., 1989), suggesting that L-glutamate could have an erastin-like effect. Dixon and colleagues also show that an iron-chelating compound suppresses L-glutamate-induced neuronal cell death in vitro. Although the evidence supporting a ferroptosis mechanism in neuronal cell death is based only on pharmacological agents with little genetic validation of the importance of the ferroptosis mechanism either in vitro or in vivo, the authors open the door for future studies by speculating that neuronal excitotoxicty and erastin-triggered cancer cell death share common features that are consistent with ferroptosis. Based on the current evidence, however, it is prudent to conclude that it remains an open question whether ferroptosis actually occurs in vivo under some pathophysiological circumstances.

An interesting feature of cell death mechanisms induced by erastin is an apparent role for lipid oxidation. Among the targets identified as enablers of erastin-induced cytoxicity of RAS-expressing tumor cells were genes encoding enzymes involved in mitochondrial fatty acid synthesis. Conversely, lipophilic antioxidant compounds were shown to be especially potent suppressors of cell death induced by erastin as well as another RAS synthetic lethal compound that selectively kills cells expressing activated RAS in an iron-dependent manner but that does not interfere with cystine transport. An intriguing possibility is that ferroptosis involves production of a specific class of cytotoxic lipids, a notion reminiscent of the requirement for certain sphingomyelin metabolites for Bax/Bak-mediated MOMP (Chipuk et al., 2012).

A conundrum in the use of erastin as a chemical probe for investigating mechanisms of ferroptosis is its reported binding to at least two putative targets: VDAC2 and the SLC7A5 subunit of amino acid

transporters. Is it necessary for erastin to bind both of these cellular targets to enable ferroptosis? Previous studies showed that erastin inhibits VDAC-mediated transport of NADH into mitochondria, thus raising the possibility that erastin's effects on VDAC may help to elevate cytosolic levels of NADPH to support the activity of cytosolic oxidases that generate lethal levels of ROS (Yagoda et al., 2007; Yang and Stockwell, 2008). Consequently, erastin might promote ROS production both by influencing the activity of NADPH-requiring oxidases and by reducing cystine-dependent glutathione production via decreased cystine transport, thereby lowering antioxidant defenses. However, given that ROS have been implicated in so many forms of cell death, one wonders whether "pure" ferroptosis is easily discerned, which is a question that would potentially yield to single-cell analysis rather than relying on bulk cell populations.

The practical utility of chemical biology efforts that have led to the discovery of compounds such as erastin, which promotes iron-dependent oxidative killing of RAS-expressing cancer cells, is yet to be determined. Oncogenic RAS mutations occur in ~20% of human cancers, but RAS itself has failed to yield to drug discovery efforts, prompting interest in downstream targets of the RAS pathway. An analog of erastin (PRLX-93936) was tested in phase I trials but was poorly tolerated, raising concerns about therapeutic index (Ramanathan et al., 2010). The FDA-approved drug Lanperisone has been reported to kill RAS-transformed tumor cells through a similar iron- and ROS-dependent mechanism (Shaw et al., 2011), suggesting a potential drug repurposing opportunity. While sulfasalazine (another approved medicine) also inhibits cystine transport, its potency is probably too weak to trigger ferroptosis in vivo. More work is needed toward understanding the mechanism(s) of ferroptosis so that optimal targets can be identified for future drug discovery efforts.

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Feeling the Squeeze: Live-Cell Extrusion Limits Cell Density in Epithelia

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Tissues develop in confined volumes that can impose mechanical constraints on their growth, but it is unclear how cells respond to these limits to regulate tissue size and shape. Two papers show that overcrowding and cell deformation lead to the shedding of live cells to maintain homeostasis in epithelial cell sheets.

How tissues acquire and maintain their size and shape is one of the mysteries of developmental biology. In nature, tissues do not develop in isolation, but in the presence of mechanical constraints imposed by their surroundings. Theoretical studies suggest that compressive forces could limit the rate of cell growth, producing a system in homeostasis in which cell proliferation