ILF and VRED Pathways Prevent Lysosome/Vacuole Rupture by Oxidative Stress

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Lysosomes are recycling centres found within all eukaryotes. Their lumenal hydrolases convert toxic, damaged or unneeded biomaterials into an important source of nutrients, which cells heavily rely on when starved. But high recycling activity comes at a cost: Because lysosomes are major stores of cellular iron, hydrogen peroxide generated by elevated hydrolytic activity is converted into reactive oxygen species (ROS) by the Fenton reaction. In turn, ROS damages proteins and lipids causing lysosome membrane permeability (LMP) allowing hydrolases to escape triggering a unique form of apoptosis called lysosomal cell death. Because cancer cells are starved in tumor environments, they require elevated recycling activity by lysosomes for survival and proliferation. But this enhanced hydrolytic activity does not trigger LMP, suggesting an unknown protective mechanism is activated to ensure cancer cell survival.

Using *Saccharomyces cerevisiae* and its vacuolar lysosome as models, we aimed to identify the cellular mechanism(s) that protect against LMP under oxidative stress (induced by adding hydrogen peroxide). We first show that two lysosomal protein degradation pathways are activated under oxidative stress – the Vacuole protein REcycling and Degradation (VRED) and IntraLumenal Fragment (ILF) pathways – to presumably clear damaged proteins (and possibly lipids) from vacuole membranes preventing rupture. Next, we find that both pathways also down-regulate two vacuole membrane proteins critical for iron homeostasis – Fth1 an iron transporter, and Fet5, an iron/copper oxidase – to presumably reduce lumenal iron and iron reactivity under oxidative stress. Consistent with these results, introducing mutations that impair either pathway enhances LMP and cell death under oxidative stress.

As the underlying mechanisms are conserved in all eukaryotes, we speculate that inhibiting the VRED or ILF pathways to promote LMP may be a new strategy to treat cancers.