Discovery of MF-0094, a potent, selective and cell permeable inhibitor of USP30

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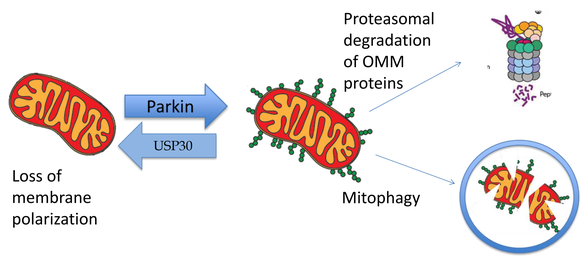
Abstract

USP30 is a ubiquitin specific protease localized to the outer mitochondrial membrane. The enzymatic activity of USP30 opposes parkin mediated ubiquitination of mitochondrial proteins, which contributes to the regulation of proteasomal degradation of specific proteins and autophagosomal destruction of the mitochondrion. A biochemical assay to detect USP30 enzyme inhibitors was developed which relied on detecting USP30 catalyzed release of rhodamine from a ubiquitin-rhodamine conjugate. HTS of a compound library identified small molecule enzyme inhibitors with IC_{50}s of less than 1 µM. These compounds were optimized for enzyme inhibition and drug-like properties; MF-0094, a USP30 inhibitor with an IC_{50} = 100 nM was identified. A minimal structural change yielded MF-0095, which has an IC_{50} > 10 µM for USP30 inhibition. These cell permeable compounds were screened for their ability to inhibit 21 other ubiquitin specific proteases and neither compound showed inhibitory activity at 10 µM. An activity-based probe, biotin-ubiquitin-vinylmethylester was found to label native USP30 on isolated mitochondria. MF-0094 inhibited the labeling whereas MF-0095 did not. Thus, USP30 dependent cellular events should be sensitive to MF-0094, and MF-0095 serves to control for any non-USP30 effects of compounds in this structural class. Consistent with the mechanistic hypothesis, MF-0094 enhances mitophagy in C2C12 myotubes whereas MF-0095 is ineffective.

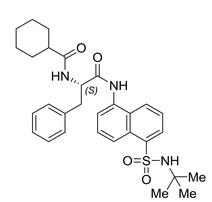
Results

Role of USP30 in mitophagy

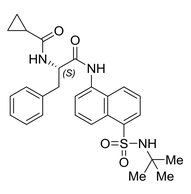


On loss of mitochondrial membrane potential the ubiquitin E3 ligase Parkin adds ubiquitin to outer membrane proteins leading to proteasomal degradation or mitophagy. USP30 catalyzes removal of ubiquitin and opposes parkin[1].

USP30 inhibitor MF-0094 and chemotype control MF-0095



MF-0094
USP30 IC_{50}= 0.11 µM



MF-0095
USP30 IC_{50} > 10µM

Figure 1: Inhibition of USP30 by MF-0094 is sensitive to modest structural changes. MF-0095 is a control for effects of the compound class that are not USP30 dependent.

Results

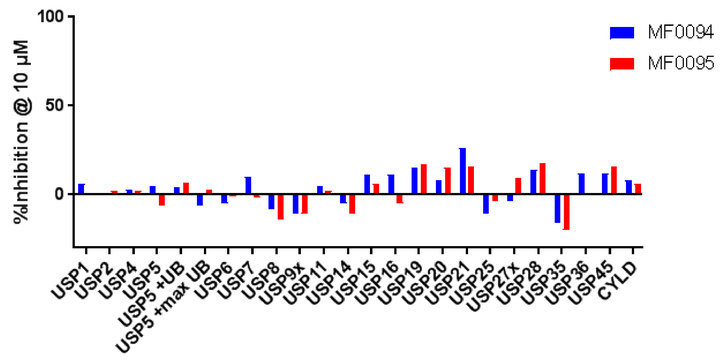
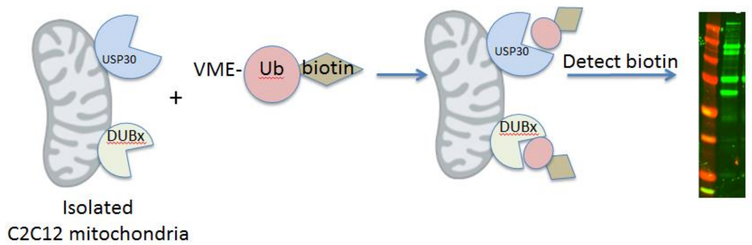


Figure 2: MF-0094 and MF-0095 inhibit USP30 specifically. Inhibition of a panel of ubiquitin specific proteases other than USP30 (Ubiquigent Ltd., Dundee, UK) shows little inhibition.

USP30 Activity Based Probe Protection Assay



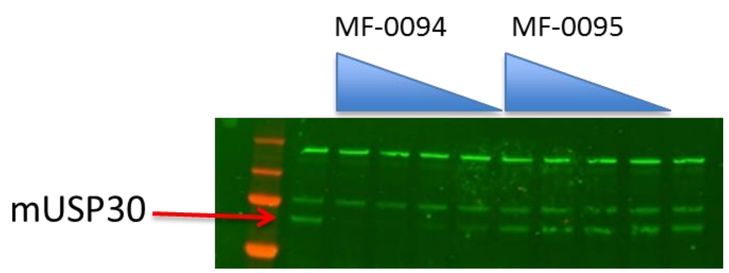
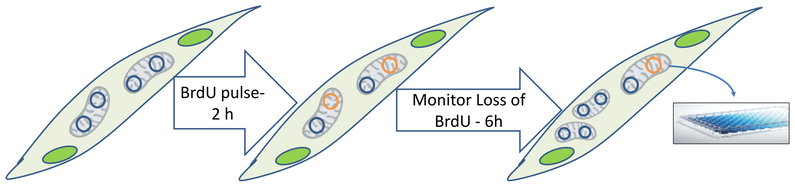


Figure 3: MF-0094 protects endogenous USP30 from labeling with the activity-based probe vinyl methylester-ubiquitin-biotin (VME-Ub-biotin; UbiQ – Amsterdam, Netherlands) on mouse mitochondria isolated from mouse C2C12 myoblasts, indicating cross species reactivity. In contrast, MF-0095 does not protect USP30 from labeling. MF-0094 is capable of inhibiting native USP30 on the mitochondrial surface. The non-USP30 bands are different deubiquitinases (DUBx) and endogenous biotin containing enzymes.

Results

C2C12 myotube Pulse/Chase Mitophagy Assay



mtDNA

C2C12 myotubes are treated with USP30 inhibitors (24h)

mtDNA – BrdU

mtDNA is labeled with bromodeoxyuridine (BrdU). No nuclear DNA replication occurs in differentiated myotubes

A BrdU ELISA detects the amount of labeled mtDNA remaining.

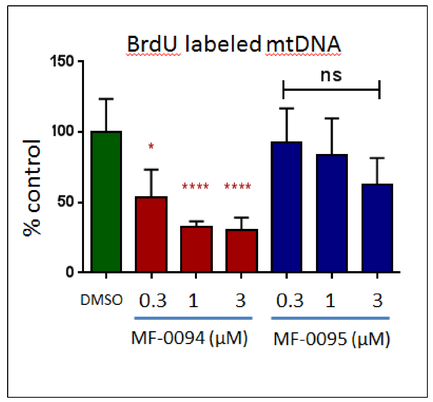


Figure 4: MF-0094 accelerates mitophagy . MF-0095 shows only nonsignificant acceleration. Neither compound affected cellular viability at the tested concentrations (not shown).

Conclusions and Future Directions

MF-0094 was initially discovered with a biochemical assay using an artificial substrate and recombinant USP30. MF-0094 is specific for USP30 compared to other USPs and inhibits native enzyme on the mitochondrial surface. Using a mitophagy assay measuring the rate of disappearance of BrdU labelled mtDNA in C2C12 myotubes, we showed that MF-0094 accelerated mitophagy but the chemotype control compound MF-0095 had no effect. MF-0094 and MF-0095 will be made available to collaborators by Mitobridge to investigate more broadly the role of USP30 in mitochondrial quality control.

Reference

[1] Bingol B *et al.* The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* **510**, 370-375 (2014).

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