Phosphorylation of TRAP1 by the tyrosine kinase c-Abl drives pro-survival signaling in renal cell carcinoma

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Background

Molecular chaperones are stress-responsive mediators of protein folding involved in pro-survival signaling[1]. The dedicated mitochondrial chaperone Tumor Necrosis Factor receptor-associated protein-1 (TRAP1) has a well-established role in the regulation of oxidative metabolism and apoptosis. Post-translational modification (PTM) is a common signal transduction mechanism and modulator of protein–protein interaction. Previous work has elucidated the complex cross-talk between PTMs that regulate many facets of chaperone function, including enzymatic activity, interaction with regulatory and dependent proteins, and druggability[2]. However, few TRAP1 PTMs have been described. TRAP1 inhibitors have been found efficacious in treating many diseases, including cancer[3-5], and understanding the PTM landscape of TRAP1 is essential to identify the appropriate cellular context in which to deploy TRAP1 inhibitors.

Programmed cell death is an essential organismal triage pathway that provides a mechanism for the steady-state clearance of both normal and dysfunctional cells. The intrinsic apoptosis pathway is controlled by the mitochondria through the activity of the permeability transition pore (PTP), and the critical effector protein Cyclophilin D (CypD). Previous work has shown that TRAP1 is an integral binding partner of CypD and inhibition of TRAP1 or CypD, or disruption of TRAP1-CypD interaction, facilitates opening of the PTP[6]. Elevated TRAP1 expression in many cancers implies an essential role for the TRAP1-mediated opposition of apoptosis in aberrant cell proliferation.

The tyrosine kinase c-Abl is a *proto*-oncogene with a demonstrated role in the regulation of cell death, though the bona fide function of c-Abl remains unknown. Previous work has shown that mitochondrial localization of c-Abl impacts mitochondrial function in a cell-type-dependent manner, however the impact of c-Abl on the PTP has not been explored[7]. It has been widely observed that in cancer cells, inhibition of either TRAP1 or c-Abl leads to apoptosis. Our preliminary data suggests that c-Abl directly phosphorylates TRAP1 within a consensus c-Abl recognition motif, justifying an examination of the role of mitochondrial c-Abl in the regulation of TRAP1-mediated cell death.

Objectives

- Characterize c-Abl–mediated phosphorylation of TRAP1.
- Determine the impact of TRAP1 phosphorylation on apoptosis.
- Observe the effect of disrupting TRAP1 phosphorylation on ccRCC survival.

TRAP1 is essential for ccRCC survival

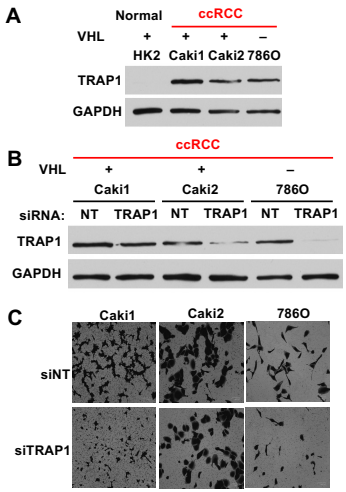


Fig. 2: TRAP1 is essential for ccRCC survival. A) Immunoblot showing TRAP1 expression in ccRCC cells compared to normal renal epithelial cells. **B)** Immunoblot of siRNA knockdown of TRAP1 in ccRCC cells. **C)** Crystal violet staining of ccRCC following non-targeting or TRAP1-targeting siRNA.

c-Abl is a mitochondrial kinase

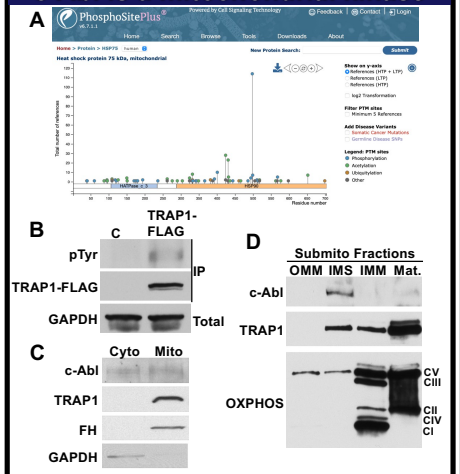


Fig. 3: c-Abl is a mitochondrial kinase. A) Compilation of TRAP1 PTM in peer-reviewed literature from PhosphoSite (Cell Signaling Tech). **B)** Immunoprecipitation of TRAP1 immunoblotted with anti-pan-phosphotyrosine. **C)** Cytosolic and mitochondrial fractions of HEK293 cells immunoblotted for c-Abl. **D)** Submitochondrial fractions of HEK293 cells demonstrate co-localization of TRAP1 and c-Abl in the intermembrane space (IMS). FH – fumarate hydratase, OMM – outer mitochondrial membrane, IMM – inner mitochondrial membrane.

c-Abl and TRAP1 cooperate to regulate apoptosis

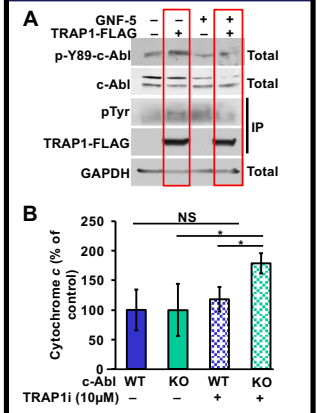


Fig 4: c-Abl and TRAP1 cooperate to regulate apoptosis. A) Immunoprecipitation of TRAP1-FLAG from HEK293 cells following treatment with the c-Abl inhibitor GNF-5. **B)** Cytochrome *c* ELISA using cytosol from wild-type and c-Abl knockout MEF cells following treatment with TRAP1-specific inhibitor Compound 5[14].

TRAP1 Chaperone Cycle

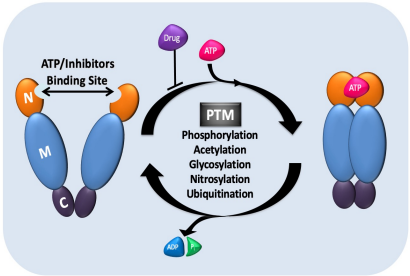
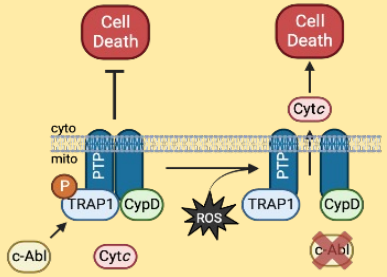


Fig 1: TRAP1 chaperone cycle. ATP binding to the N-terminal domains of TRAP1 promotes a series of conformational rearrangements. Subsequent structural changes result in the 'closed and twisted' conformation of TRAP1 that is capable of ATP hydrolysis and chaperoning of "client" proteins[8-13]. This cycle can be disrupted by small molecule ATP-competitive inhibitors. The chaperone cycle of the related Hsp90 chaperone is regulated by diverse post-translational modifications (PTMs), however very few TRAP1 PTMs have been identified.

TRAP1 phosphorylation by c-Abl drives pro-survival signaling in ccRCC



TRAP1 and c-Abl converge on the regulation of the PTP and subsequent mitochondrial apoptosis. TRAP1 and CypD interaction maintains PTP in closed state. TRAP1 phosphorylation by c-Abl promotes this interaction. Disruption of TRAP1-CypD interaction, inhibition of c-Abl, or elevated ROS can drive PTP opening, cytochrome *c* release, and apoptosis.

Q: How are signals communicated to TRAP1 to regulate apoptosis?

A: c-Abl–mediated phosphorylation.

Premise: TRAP1 is a mitochondrial chaperone known to regulate mitochondrial apoptosis. Cancers are sensitive to inhibition of either c-Abl or TRAP1.

Findings: TRAP1 is phosphorylated by c-Abl in the mitochondria, which drives maintenance of the PTP. Combined attenuation of TRAP1 and c-Abl leads to elevated apoptosis.

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Ask a question!

Future Directions

- Characterize c-Abl/TRAP1 axis in ccRCC
- Identify impact of c-Abl phosphorylation on TRAP1-CypD complex
- Evaluate potential co-targeting of TRAP1 and c-Abl in ccRCC

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Acknowledgements

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