Impact of TRAP1 on Cancer Metabolism

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The Hsp90 chaperone TNF-receptor-associated protein-1 (TRAP1) is primarily localized to the mitochondria and controls both cellular metabolic reprogramming and mitochondrial apoptosis. TRAP1 upregulation facilitates the growth and progression of many cancers by promoting glycolytic metabolism and antagonizing the mitochondrial permeability transition that precedes multiple cell death pathways. TRAP1 attenuation induces apoptosis in cellular models of cancer, identifying TRAP1 as a potential therapeutic target in cancer. Similar to cytosolic Hsp90 proteins, TRAP1 is also subject to post-translational modifications (PTM) that regulate its function and mediate its impact on downstream effectors, or 'clients'.

TRAP1 Hsp90 chaperone post-translational modification cancer mitochondria metabolism Warburg effect

1. Introduction

Molecular chaperones of the heat shock protein-90 (Hsp90) family are involved in signal integration and the cellular stress response. These chaperones mediate cell signaling through the stabilization and activation of their substrate proteins, known as clients (https://www.picard.ch/downloads/Hsp90interactors.pdf, accessed 28 February 2022) [1]. The Hsp90 chaperone function is coupled to the ability to hydrolyze ATP, and chaperone activity can be precisely regulated by a heterogeneous group of proteins known as co-chaperones [2], as well as a diverse array of post-translational modifications (PTM) [3].

TNF-receptor-associated protein-1 (TRAP1) is the mitochondrial-dedicated Hsp90 family member and is localized to the mitochondrial matrix, inner mitochondrial membrane, and the intermembrane space [4][5][6]. TRAP1 was first identified through its interaction with the intracellular domain of the Type I TNF receptor [7], and early characterization of TRAP1 demonstrated ATP-binding ability and sensitivity to ATP-competitive Hsp90 inhibitors [8]. Despite this, TRAP1 was unable to form complexes with known cytosolic Hsp90 co-chaperones, nor could it promote the maturation of Hsp90 client proteins, suggesting a distinct mechanism of action for TRAP1 [8].

From this time, work has concentrated on the impact of TRAP1 on cellular processes, however identification of TRAP1 effectors and regulatory mechanisms of TRAP1 expression and activity are critical to understanding its biological function. TRAP1 has an established role as a master regulator of metabolic flux, and a large body of evidence has demonstrated that TRAP1 expression serves to suppress oxidative phosphorylation [9][10][11]. Further, TRAP1 also contributes to cell survival through complex formation with cyclophilin D (CypD), which regulates the

opening of the permeability transition pore (PTP) [12]. These two known roles suggest a critical function for TRAP1 in maintaining cellular homeostasis [13].

2. Structural Basis of TRAP1 Activity

Hsp90 family chaperones are characterized by their dimeric structure. Each of the two protomers are composed of an amino-terminal ATP-binding domain, followed by a middle domain, the primary interface for client interaction, and a C-terminal domain that allows constitutive dimerization of the protomers [14]. Hsp90 chaperone activity is coupled to its ability to hydrolyze ATP [15][16]. The ‘chaperone cycle’ begins with ATP binding to the ‘open’ conformation of Hsp90, followed by transient dimerization of the N-terminal domains of each protomer and ATP hydrolysis, and subsequent release of mature client proteins and regeneration of the ‘open’ Hsp90 dimer [17]. TRAP1 is broadly structurally similar to cytosolic Hsp90, with some notable exceptions, including a cleavable N-terminal mitochondrial localization signal and an N-terminal extension or ‘strap’ that stabilizes the ‘closed’ conformation of TRAP1 [18][19]. Asymmetrical post-translational modification and co-chaperone binding are important determinants of Hsp90 molecular chaperone function [18][20][21][22][23][24]. Interestingly, TRAP1 dimers are inherently asymmetric, and uniquely composed of one ‘straight’ and one ‘buckled’ protomer, with the buckled protomer demonstrating increased rates of ATP hydrolysis [25] (**Figure 1**). Recently, structural and cell-based studies have described a tetrameric form of TRAP1 induced in response to dysregulation of oxidative metabolism, although the impact of this TRAP1 state on its activity is as yet unknown [26]. Interestingly, whether TRAP1 ATPase activity is essential for the entire scope of its biological role also remains an open question [26].

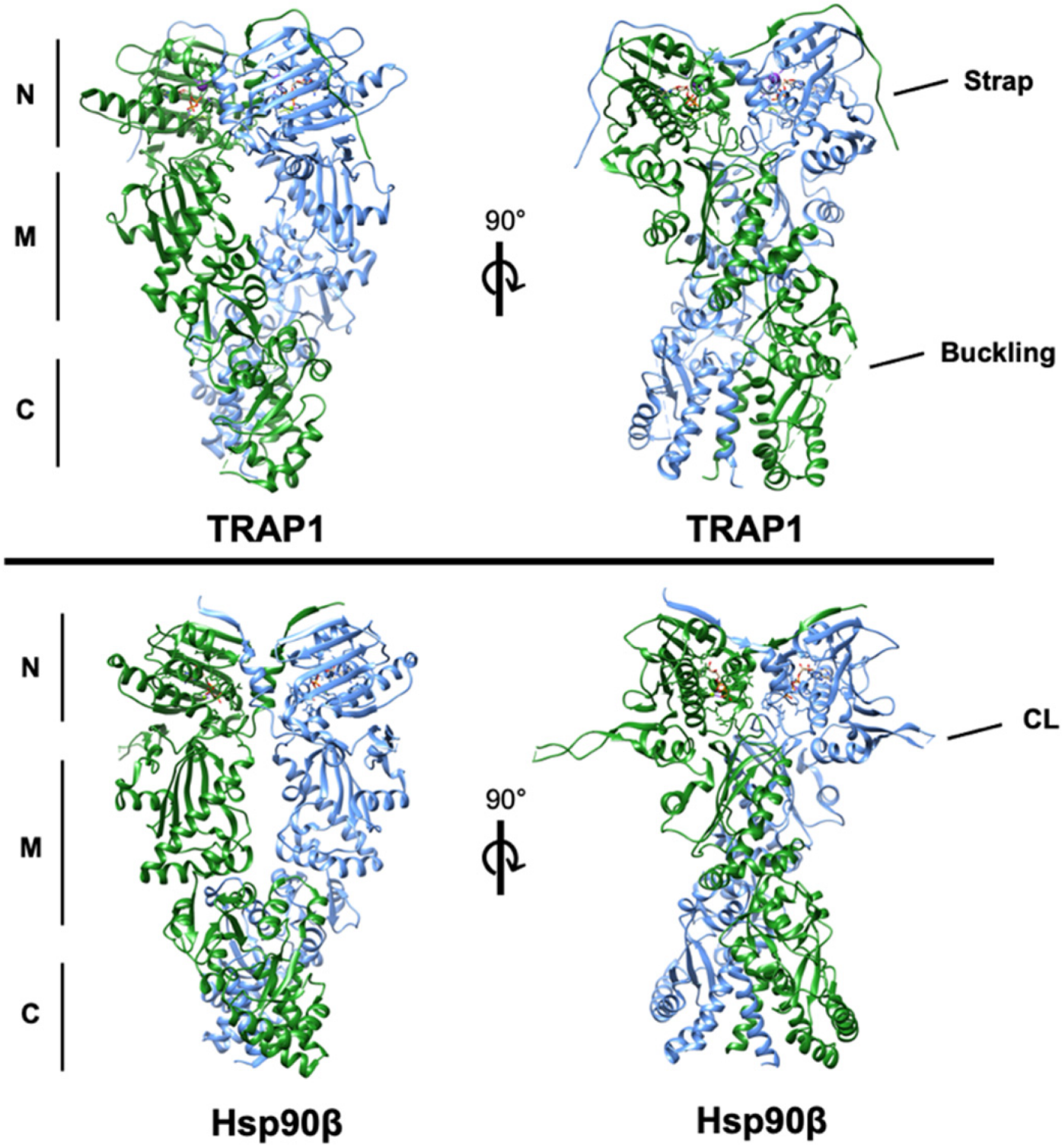

Figure 1. Structures of human TRAP1 (PDB: 6xg6) and human Hsp90β (PDB: 5fwp) bound to nucleotide with the conserved N-, middle-, and C-domains denoted. One protomer of each is colored blue and the second is colored green. The regulatory N-terminal extension (strap) of each TRAP1 protomer can be observed overlapping the opposite protomer. The region of TRAP1 near the M-C boundary that 'buckles' during conformational rearrangement is incompletely resolved in the structure. Additionally, the resolved residues of the charged linker domain (CL) of cytosolic Hsp90, which is absent in TRAP1, are labeled in the lower right quadrant.

3. Impact of TRAP1 on Cancer Metabolism

Controversially, TRAP1 has alternately been characterized as an oncogene and tumor suppressor, and it has been suggested that TRAP1 is essential for malignant transformation of cells but dispensable at later stages of tumor development [6][27]. Despite this controversy, much of the literature supports the idea that TRAP1 regulates metabolic transformation during tumorigenesis, TRAP1 is overexpressed in many cancers, and TRAP1 attenuation is detrimental to tumor cell survival [28][29][30][31][32][33]. It may be more appropriate to suggest that, similar to cytosolic Hsp90, many cancers may be 'addicted' to TRAP1 [34][35][36]. In fact, multiple pathways in which TRAP1 activity can drive tumorigenesis have been described (**Figure 2**).

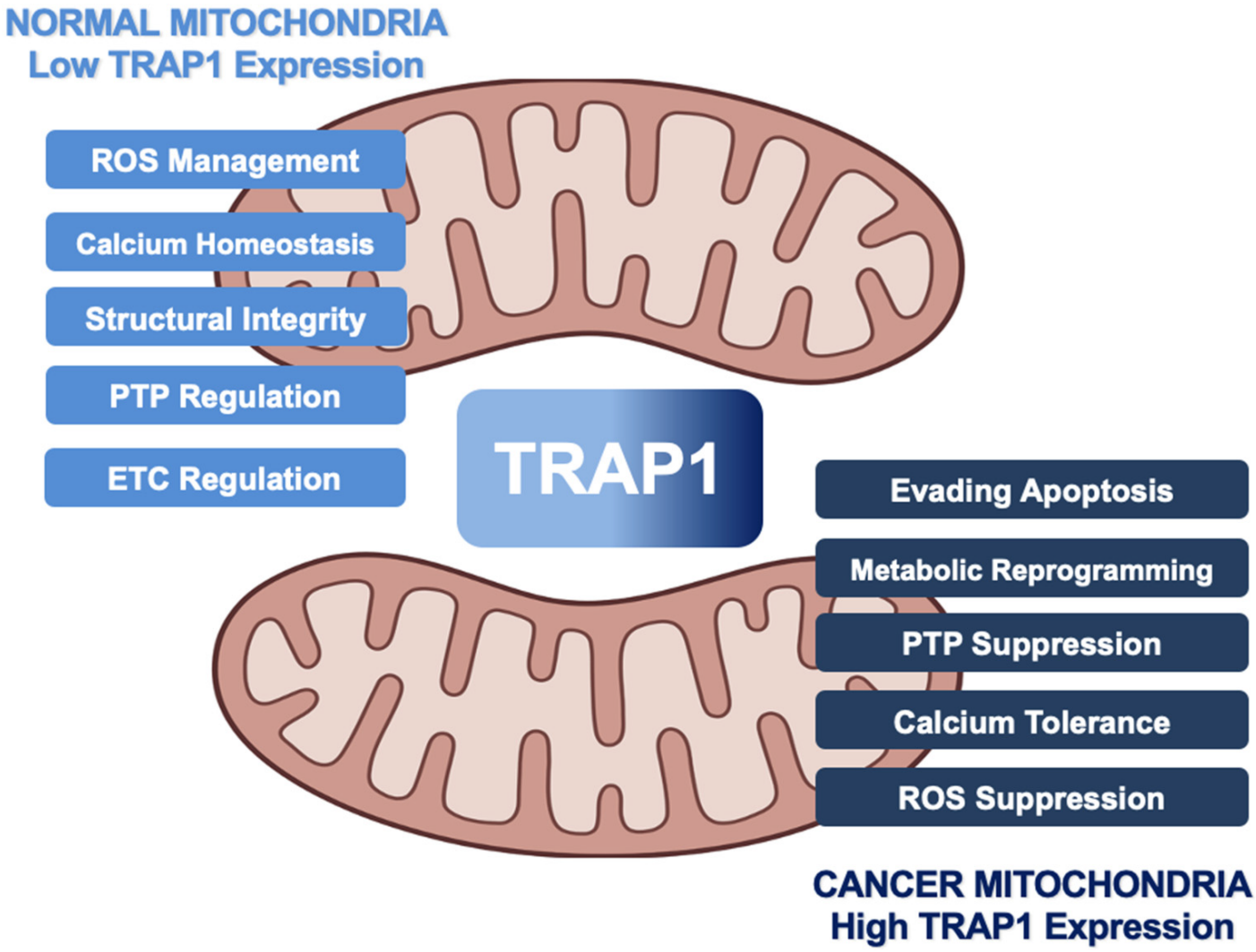

Figure 2. Role of human TRAP1 in mitochondria of normal cells and cancer cells. Normal expression levels (light blue) lead to TRAP1 regulation of ROS and calcium levels, integrity of cristae, function of ETC, and oversight of the PTP. As TRAP1 expression increases (dark blue), mitochondria lose calcium sensitivity, downregulate ROS, and prevent PTP opening, leading to metabolic reprogramming and evasion of apoptosis in cancer.

3.1. Metabolic Regulation

The cellular energy currency adenosine triphosphate (ATP) is generated as a consequence of the complete oxidation of glucose to CO_2 and H_2O, and each molecule of glucose can maximally result in 36–38 ATP molecules [37]. Normal cells produce ATP primarily through cellular respiration, which describes a process in which glucose metabolism by glycolysis is coupled to the tricarboxylic acid cycle (TCA). Concurrent mitochondrial electron transport generates the electrochemical gradient that provides the force by which ATP is disseminated throughout the cell [38]. ATP generation is highly dysregulated in cancers, and many cancer subtypes supplement their ATP supply by upregulating cytosolic glycolysis, simultaneously generating additional ATP driven by the terminal fermentation of pyruvate to lactate [39]. This hyperactive glycolytic phenotype is known as the Warburg effect, and serves to support the accelerated growth of cancers through the increased synthesis of intermediates for anaplerotic metabolism and hypertrophy [40][41]. The phenotypic manifestations of metabolic dysregulation are variable and dependent on cell type and genotype, and many of the details and nuances of this differential regulation remain obscured.

Few specific biological roles and binding partners have been described for TRAP1, despite the broad understanding of its impact on metabolic flux. Two of the few described bona fide clients of TRAP1 however are subunits of electron transport chain (ETC) complexes, Complex II components succinate dehydrogenase subunit A/B (SDHA/B) [42][43][44][45], and Complex IV cytochrome *c* oxidase subunit 2 (COXII) [6][46][47]. Complex II/SDH is an iron–sulfur cluster-containing protein complex that functions to transfer electrons from succinate to coenzyme Q10-ubiquinone (Complex III) [48]. In agreement with the understanding of Hsp90 function, TRAP1 maintains SDH in a partially unfolded state [49], and TRAP1 inhibition releases active SDH, leading to an increase in its activity [27][44][50][51][52]. Further, SDH activity [44][53][54] and the oxygen consumption rate [6][55] are inversely correlated with TRAP1 expression, implicating TRAP1 in promoting the Warburg effect [56]. Notably, SDH also oxidizes succinate to fumarate and thus integrates the TCA cycle and the ETC, indicative of the broad influence of TRAP1 on mitochondrial metabolism [56][57][58].

Complex IV of the ETC converts molecular oxygen to water, and in doing so enacts the final step in generating the electrochemical gradient that supports ATP production by Complex V (ATP synthase) [59]. COXII is a downstream effector of TRAP1 function in the regulation of apoptosis, and TRAP1 regulates COXII expression [47] and activity [6]. As downregulation or inhibition of TRAP1 has been shown to destabilize COXII [46][50] and deletion of TRAP1 was associated with decreased COXIV subunit levels [60], it is possible that TRAP1 chaperoning of COXII/IV is mechanistically similar to SDHA/B. TRAP1 has also been shown to interact with the Complex V subunit ATPB, although little is known about this interaction [27].

Mitochondrial respiration drives the production of reactive oxygen species (ROS) and is responsible for most cellular ROS (**Figure 3**) [61]. In considering the role of TRAP1 in chaperoning SDH and COXII, TRAP1-mediated regulation of mitochondrial respiration suppresses ROS production [62], thereby contributing to the regulation of redox homeostasis, metabolic flux, and mitochondrial apoptosis.

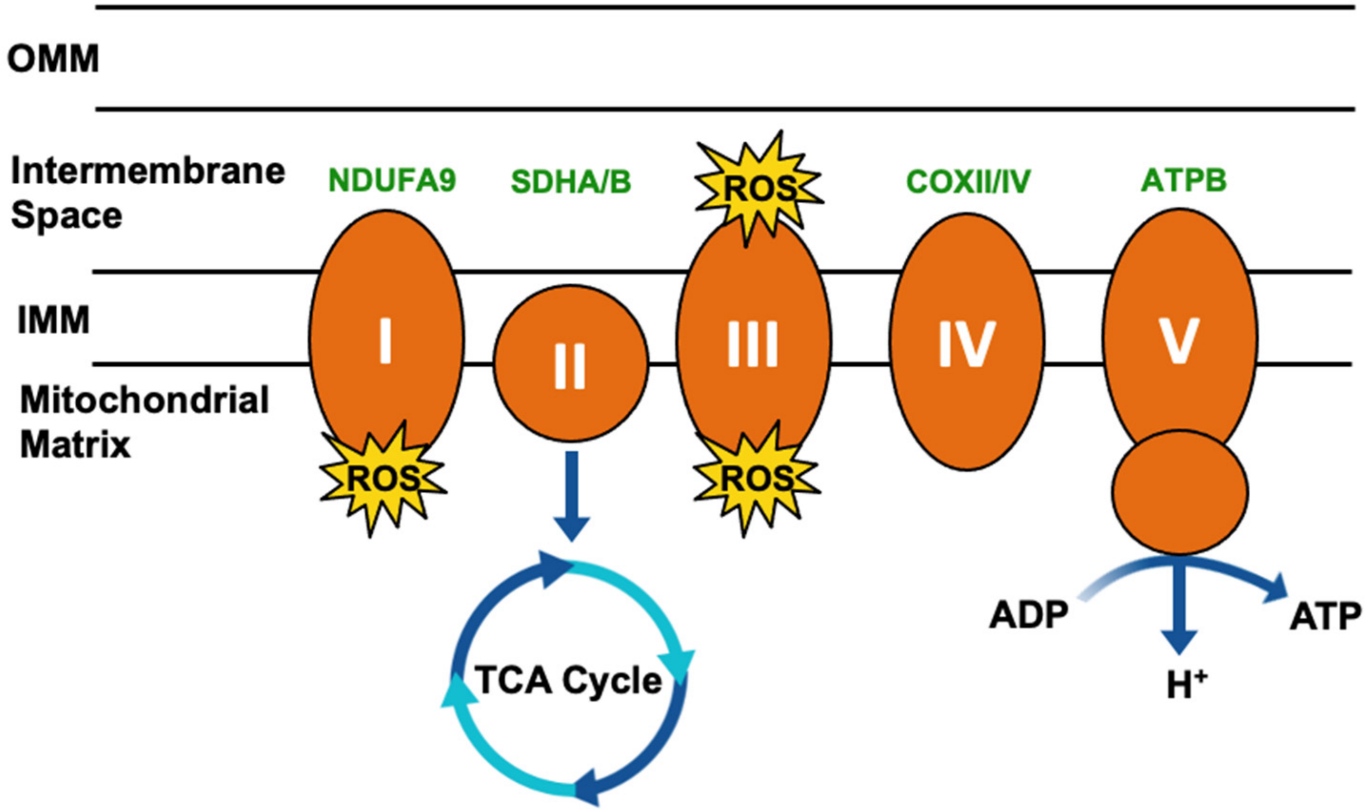

Figure 3. Simplified mitochondrial respiration schematic. Electron transport chain (ETC) complexes (I–V) are represented by orange ovals, and reactive oxygen species (ROS) generated as a byproduct of Complex I and III activity is represented by yellow starbursts. Succinate dehydrogenase (SDH)/Complex II connects the ETC to the tricarboxylic acid (TCA) cycle. TRAP1 interactors involved in this process have been highlighted in green.

3.2. Contribution to Tumorigenesis

Cancer-associated increases in TRAP1 expression suggest a role for TRAP1 in oncogenesis [30][63][64]. Indeed, TRAP1 deletion delayed tumor formation in a mouse model of breast cancer, providing direct evidence of the role of TRAP1 in tumor initiation [65]. Further, TRAP1-mediated SDH inhibition leads to accumulation of the oncometabolite succinate [58]. Increased succinate inhibits the activity of prolyl hydroxylases, which are responsible for the hydroxylation of the transcription factor hypoxia inducible factor (HIF1α), a prerequisite for recognition by the VHL-dependent E3-ubiquitin ligase machinery [66]. Succinate-dependent HIF1α stabilization and activation promotes a well-established glycolytic transcriptional program [67], demonstrating yet another function of TRAP1 in the regulation of cancer-associated metabolic dysregulation.

TRAP1 expression was found to be elevated in aggressive pre-neoplastic lesions in a rat model of hepatocarcinogenesis [68]. The master antioxidant transcription factor NRF2 was also activated in this model, and given the established role of TRAP1 in regulating intracellular ROS, TRAP1 likely participates in NRF2-driven ROS mitigation during tumor development [68]. NRF2 inhibition led to decreased TRAP1 levels independent of TRAP1 transcription [68], suggesting that post-translational regulation is essential for sustained TRAP1 expression in pre-cancerous and cancerous cells. Interestingly, pentose phosphate pathway (PPP) flux was found to be increased in

this model, and was determined to be a consequence of elevated citrate synthase activity in aggressive pre-neoplastic lesions [68]. Citrate accumulation inhibits downstream metabolic enzymes phosphofructokinase and SDH and activates the anaplerotic PPP [69]. This increase in citrate synthase activity was alleviated following TRAP1 knockdown or inhibition, suggesting that citrate synthase may also be a TRAP1 client [68].

Cell cycle dysregulation is a well-established driver of tumorigenesis [70]. TRAP1 impacts the cell cycle through regulation of protein quality control in cooperation with the proteasome regulator TBP7 [71][72]. Loss of the TRAP1/TBP7 machinery leads to increased ubiquitination and degradation of the G2-M checkpoint proteins CDK1 and MAD2 and dysregulation of mitotic entry [72]. However, whether TBP7 is a client or perhaps even the first co-chaperone of TRAP1 remains to be seen.

Taken together, these data describe multiple mechanisms through which TRAP1 dysregulation can impact cellular metabolic flux and, potentially, tumorigenesis.

3.3. Evasion of Apoptosis

Mitochondrial involvement in cell death is mediated by the release of cytochrome *c* [73][74]. Sustained opening of the permeability transition pore (PTP) within the inner mitochondrial membrane (IMM) initiates a series of events that lead to cytochrome *c* release and apoptosis or necrosis. Upon PTP opening, particles under 1500 Da, such as ions (Ca^{2+}, K^{+}, and H^{+}), water, and other solutes, flood the IMM, causing swelling and unfolding of the cristae and eventual outer mitochondrial membrane (OMM) rupture. Subsequent efflux of cytochrome *c* through the compromised OMM into the cytosol induces the caspase cascade [75][76]. This sustained PTP opening is known as the mitochondrial permeability transition (PT) [77], and it can be triggered by several mechanisms, including elevated ROS, Ca^{2+}, or inorganic phosphate levels, as well as decreased pH or ATP depletion [78]. Interplay between these elements also plays a role in its regulation, as elevated ROS has been shown to decrease the amount of Ca^{2+} required to trigger the PTP [76].

TRAP1 attenuation induces opening of the PTP and release of cytochrome *c* [47], and expression of TRAP1 likely discourages the initiation of apoptosis through two distinct, but potentially overlapping mechanisms: (1) regulation of triggers that signal into the PTP, and (2) direct disruption of the physical mechanism of PTP opening. TRAP1 knockdown has been shown to lead to increased ROS accumulation under oxidative stress [79] and TRAP1 overexpression insulates cells against iron chelation-mediated ROS production [80]. These effects are likely a consequence of both direct and indirect roles of TRAP1 in minimizing ROS generation. TRAP1 is a direct regulator of oxidative phosphorylation through its chaperoning of Complexes II and IV of the ETC [6][44][46] and has an indirect role in quenching existing ROS, as TRAP1 expression is associated with increased levels of the reduced form of the antioxidant glutathione (GSH) [81]. TRAP1-dependent regulation of ROS generation also results in decreased oxidation of the phospholipid cardiolipin. This phospholipid is responsible for the binding of cytochrome *c* to the inner folds of cristae, and its oxidation results in an increase of free cytochrome *c* in the inner membrane space that can potentially escape into the cytosol [78].

Furthermore, TRAP1 has been shown to chaperone the calcium-binding protein Sorcin [82]. TRAP1 is also thought to be responsible for Sorcin translocation into the mitochondria, given that Sorcin lacks its own mitochondrial localization sequence [8][82]. Overexpression of Sorcin in neonatal cardiac myocytes has been shown to increase mitochondrial Ca^{2+} levels, while simultaneously decreasing cytochrome *c* release, indicating an increase in mitochondrial Ca^{2+} tolerance [83]. Therefore, the chaperoning of Sorcin by TRAP1 is important for desensitizing the PTP to Ca^{2+} levels. Understanding this regulation is particularly important for TRAP1, as Ca^{2+} can replace Mg^{2+} as a co-factor and induce an increased rate of TRAP1 ATP hydrolysis [84]. TRAP1 has also been shown to decrease ubiquitination of the mitochondrial contact site and cristae organizing system subunit 60 (MIC60) under conditions of extracellular acidosis [85]. MIC60 is a critical component of the protein complex MICOS, which is regarded as the master organizer of the IMM through the formation of contact sites with the outer membrane and maintenance of cristae junctions [86][87]. Thus, TRAP1 regulation of MIC60 contributes to its anti-apoptotic function through the preservation of mitochondrial integrity.

Proposals for the structure of the PTP have gone through various iterations, however the prevailing model is that the PTP is formed by coordinated activities of the adenine nucleotide translocator (ANT) and the F-ATP synthase [88][89][90]. Furthermore, cyclophilin D (CypD) is key to PTP regulation [12][91]. Though its role in this process is controversial, CypD peptidyl-prolyl isomerase activity is required, as is its binding to the mitochondrial peripheral stalk subunit of the F-ATP synthase [63][90][92]. In addition to attenuating the triggers that lead to PTP opening, TRAP1 has been shown to antagonize the opening of the PTP itself. There is a general consensus that TRAP1 accomplishes this by forming a complex with CypD, interfering with the ability of CypD to interact with the PTP [12][63][93] potentially at the peripheral stalk of F-ATP synthase [90].

Further, the mitochondrial chaperones Hsp60 and Hsp90 have been implicated in this process, as their association with CypD also prevents PTP opening; however, the architecture of this complex has yet to be characterized [12][63][93][94][95][96].

4. Post-Translational Regulation of TRAP1

Post-translational modification is critically important to mitochondrial function [97] and has previously been shown to regulate TRAP1, though relatively little is known about individual PTM sites (**Table 1**, **Figure 4**) [5][6][98][99]. A comprehensive study of cytosolic Hsp90 has demonstrated the importance of post-translational regulation to Hsp90 chaperone activity (reviewed in [3][100]), and in the absence of certain co-chaperone regulatory proteins, specific PTM events have been shown to functionally recapitulate their activity [101]. This phenomenon may be critically important for TRAP1 biology, as TRAP1 is thought to act without the assistance of co-chaperones [8][10].

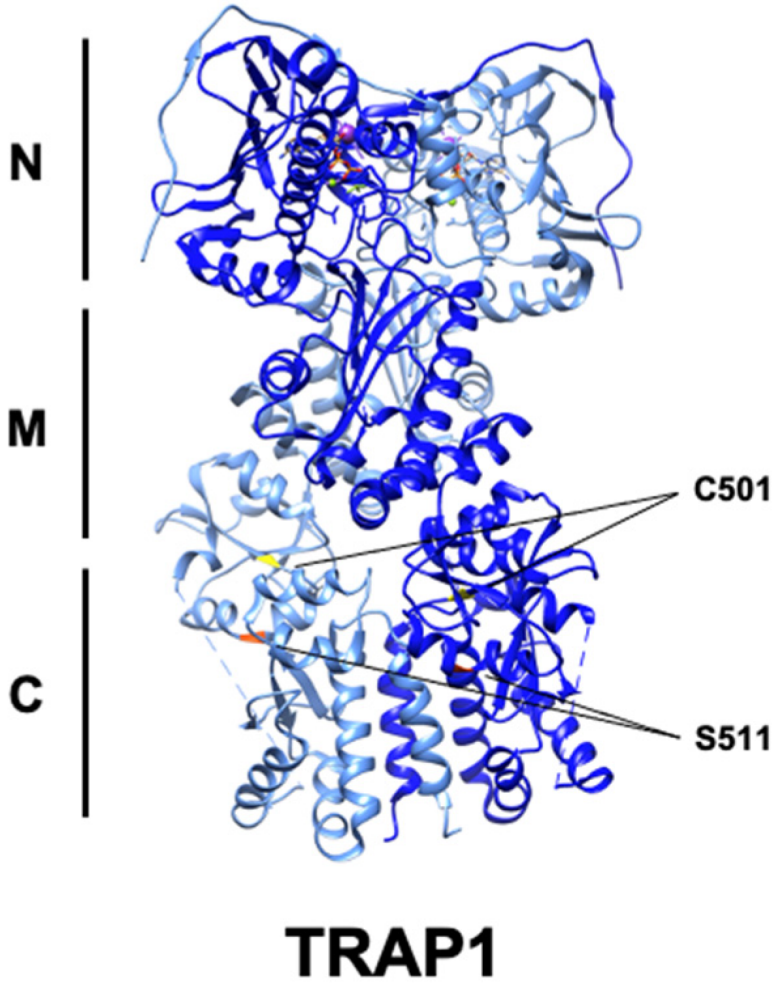

Figure 4. Ribbon structure of human TRAP1 (PDB: 6xg6) with known PTM sites. C501 (yellow) and S511 (red) are highlighted, while S568 is absent.

Table 1. Reported PTMs of TRAP1. Paralog identifies conserved residues in Hsp90α. GSNOR—S-nitrosoglutathione reductase, ERK—extracellular signal-regulated kinase.

Modification	Enzyme	Residue	Paralog	Impact on TRAP1	Reference
S-Nitrosylation	GSNOR	Cys501	Thr495	Decreased activity, proteasomal degradation	[98]
Phosphorylation	ERK1/2	Ser511	Ser505	N/A	[10]
Phosphorylation	ERK1/2	Ser568	Glu562	Increased SDH inhibition	[10]

Modification	Enzyme	Residue	Paralog	Impact on TRAP1	Reference
S/T Phosphorylation	PINK1	N/A	N/A	N/A	[5]
Y Phosphorylation	Unknown, possibly c-Src	N/A	N/A	Disrupts c-Src interaction	[6]
Deacetylation	SIRT3	N/A	N/A	Increased activity	[27]

References

1. Prodromou, C.; Bjorklund, D.M. Advances towards Understanding the Mechanism of Action of the Hsp90 Complex. Biomolecules 2022, 12, 600.
2. Johnson, J.L. Mutations in Hsp90 Cochaperones Result in a Wide Variety of Human Disorders. Front. Mol. Biosci. 2021, 8, 787260.
3. Backe, S.J.; Sager, R.A.; Woodford, M.R.; Makedon, A.M.; Mollapour, M. Post-Translational Modifications of Hsp90 and Translating the Chaperone Code. J. Biol. Chem. 2020, 295, 11099–11117.
4. Cechetto, J.D.; Gupta, R.S. Immunoelectron Microscopy Provides Evidence That Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP-1) Is a Mitochondrial Protein Which Also Localizes at Specific Extramitochondrial Sites. Exp. Cell Res. 2000, 260, 30–39.
5. Pridgeon, J.W.; Olzmann, J.A.; Chin, L.-S.; Li, L. PINK1 Protects against Oxidative Stress by Phosphorylating Mitochondrial Chaperone TRAP1. PLoS Biol. 2007, 5, e172.
6. Yoshida, S.; Tsutsumi, S.; Muhlebach, G.; Sourbier, C.; Lee, M.-J.; Lee, S.; Vartholomaiou, E.; Tatokoro, M.; Beebe, K.; Miyajima, N.; et al. Molecular Chaperone TRAP1 Regulates a Metabolic Switch between Mitochondrial Respiration and Aerobic Glycolysis. Proc. Natl. Acad. Sci. USA 2013, 110, E1604–E1612.
7. Song, H.Y.; Dunbar, J.D.; Zhang, Y.X.; Guo, D.; Donner, D.B. Identification of a Protein with Homology to Hsp90 That Binds the Type 1 Tumor Necrosis Factor Receptor. J. Biol. Chem. 1995, 270, 3574–3581.
8. Felts, S.J.; Owen, B.A.L.; Nguyen, P.; Trepel, J.; Donner, D.B.; Toft, D.O. The Hsp90-Related Protein TRAP1 Is a Mitochondrial Protein with Distinct Functional Properties. J. Biol. Chem. 2000, 275, 3305–3312.

9. Cannino, G.; Ciscato, F.; Masgras, I.; Sánchez-Martín, C.; Rasola, A. Metabolic Plasticity of Tumor Cell Mitochondria. Front. Oncol. 2018, 8, 333.
10. Masgras, I.; Ciscato, F.; Brunati, A.M.; Tibaldi, E.; Indraccolo, S.; Curtarello, M.; Chiara, F.; Cannino, G.; Papaleo, E.; Lambrughi, M.; et al. Absence of Neurofibromin Induces an Oncogenic Metabolic Switch via Mitochondrial ERK-Mediated Phosphorylation of the Chaperone TRAP1. Cell Rep. 2017, 18, 659–672.
11. Masgras, I.; Laquatra, C.; Cannino, G.; Serapian, S.A.; Colombo, G.; Rasola, A. The Molecular Chaperone TRAP1 in Cancer: From the Basics of Biology to Pharmacological Targeting. Semin. Cancer Biol. 2021, 76, 45–53.
12. Porter, G.A.; Beutner, G. Cyclophilin D, Somehow a Master Regulator of Mitochondrial Function. Biomolecules 2018, 8, 176.
13. Altieri, D.C.; Stein, G.S.; Lian, J.B.; Languino, L.R. TRAP-1, the Mitochondrial Hsp90. Biochim. Et Biophys. Acta (BBA)-Mol. Cell Res. 2012, 1823, 767–773.
14. Schopf, F.H.; Biebl, M.M.; Buchner, J. The HSP90 Chaperone Machinery. Nat. Rev. Mol. Cell Biol. 2017, 18, 345–360.
15. Obermann, W.M.J.; Sondermann, H.; Russo, A.A.; Pavletich, N.P.; Hartl, F.U. In Vivo Function of Hsp90 Is Dependent on ATP Binding and ATP Hydrolysis. J. Cell Biol. 1998, 143, 901–910.
16. Panaretou, B. ATP Binding and Hydrolysis Are Essential to the Function of the Hsp90 Molecular Chaperone Invivo. EMBO J. 1998, 17, 4829–4836.
17. Sahasrabudhe, P.; Rohrberg, J.; Biebl, M.M.; Rutz, D.A.; Buchner, J. The Plasticity of the Hsp90 Co-Chaperone System. Mol. Cell 2017, 67, 947–961.e5.
18. Lavery, L.A.; Partridge, J.R.; Ramelot, T.A.; Elnatan, D.; Kennedy, M.A.; Agard, D.A. Structural Asymmetry in the Closed State of Mitochondrial Hsp90 (TRAP1) Supports a Two-Step ATP Hydrolysis Mechanism. Mol. Cell 2014, 53, 330–343.
19. Partridge, J.R.; Lavery, L.A.; Elnatan, D.; Naber, N.; Cooke, R.; Agard, D.A. A Novel N-Terminal Extension in Mitochondrial TRAP1 Serves as a Thermal Regulator of Chaperone Activity. eLife 2014, 3, e03487.
20. Ali, M.M.U.; Roe, S.M.; Vaughan, C.K.; Meyer, P.; Panaretou, B.; Piper, P.W.; Prodromou, C.; Pearl, L.H. Crystal Structure of an Hsp90–Nucleotide–P23/Sba1 Closed Chaperone Complex. Nature 2006, 440, 1013–1017.
21. Mayer, M.P.; Le Breton, L. Hsp90: Breaking the Symmetry. Mol. Cell 2015, 58, 8–20.
22. Mollapour, M.; Bourboulia, D.; Beebe, K.; Woodford, M.R.; Polier, S.; Hoang, A.; Chelluri, R.; Li, Y.; Guo, A.; Lee, M.-J.; et al. Asymmetric Hsp90 N Domain SUMOylation Recruits Aha1 and ATP-Competitive Inhibitors. Mol. Cell 2014, 53, 317–329.

23. Retzlaff, M.; Hagn, F.; Mitschke, L.; Hessling, M.; Gugel, F.; Kessler, H.; Richter, K.; Buchner, J. Asymmetric Activation of the Hsp90 Dimer by Its Cochaperone Aha1. Mol. Cell 2010, 37, 344–354.
24. Vaughan, C.K.; Gohlke, U.; Sobott, F.; Good, V.M.; Ali, M.M.U.; Prodromou, C.; Robinson, C.V.; Saibil, H.R.; Pearl, L.H. Structure of an Hsp90-Cdc37-Cdk4 Complex. Mol. Cell 2006, 23, 697–707.
25. Elnatan, D.; Betegon, M.; Liu, Y.; Ramelot, T.; Kennedy, M.A.; Agard, D.A. Symmetry Broken and Rebroken during the ATP Hydrolysis Cycle of the Mitochondrial Hsp90 TRAP1. eLife 2017, 6, e25235.
26. Joshi, A.; Dai, L.; Liu, Y.; Lee, J.; Ghahhari, N.M.; Segala, G.; Beebe, K.; Jenkins, L.M.; Lyons, G.C.; Bernasconi, L.; et al. The Mitochondrial HSP90 Paralog TRAP1 Forms an OXPHOS-Regulated Tetramer and Is Involved in Mitochondrial Metabolic Homeostasis. BMC Biol. 2020, 18, 10.
27. Park, H.-K.; Hong, J.-H.; Oh, Y.T.; Kim, S.S.; Yin, J.; Lee, A.-J.; Chae, Y.C.; Kim, J.H.; Park, S.-H.; Park, C.-K.; et al. Interplay between TRAP1 and Sirtuin-3 Modulates Mitochondrial Respiration and Oxidative Stress to Maintain Stemness of Glioma Stem Cells. Cancer Res 2019, 79, 1369–1382.
28. Lisanti, S.; Garlick, D.S.; Bryant, K.G.; Tavecchio, M.; Mills, G.B.; Lu, Y.; Kossenkov, A.V.; Showe, L.C.; Languino, L.R.; Altieri, D.C. Transgenic Expression of the Mitochondrial Chaperone TNFR-Associated Protein 1 (TRAP1) Accelerates Prostate Cancer Development. J. Biol. Chem. 2016, 291, 25247–25254.
29. Ramkumar, B.; Dharaskar, S.P.; Mounika, G.; Paithankar, K.; Sreedhar, A.S. Mitochondrial Chaperone, TRAP1 as a Potential Pharmacological Target to Combat Cancer Metabolism. Mitochondrion 2020, 50, 42–50.
30. Si, T.; Yang, G.; Qiu, X.; Luo, Y.; Liu, B.; Wang, B. Expression of Tumor Necrosis Factor Receptor-Associated. Int. J. Clin. Exp. Pathol. 2015, 8, 13090–13095.
31. Zhang, B.; Wang, J.; Huang, Z.; Wei, P.; Liu, Y.; Hao, J.; Zhao, L.; Zhang, F.; Tu, Y.; Wei, T. Aberrantly Upregulated TRAP1 Is Required for Tumorigenesis of Breast Cancer. Oncotarget 2015, 6, 44495–44508.
32. Xie, S. The Mitochondrial Chaperone TRAP1 as a Candidate Target of Oncotherapy. Front. Oncol. 2021, 10, 11.
33. Serapian, S.A.; Sanchez-Martín, C.; Moroni, E.; Rasola, A.; Colombo, G. Targeting the Mitochondrial Chaperone TRAP1: Strategies and Therapeutic Perspectives. Trends Pharmacol. Sci. 2021, 42, 566–576.

34. Miyata, Y.; Nakamoto, H.; Neckers, L. The Therapeutic Target Hsp90 and Cancer Hallmarks. Curr. Pharm. Des. 2013, 19, 347–365.
35. Trepel, J.; Mollapour, M.; Giaccone, G.; Neckers, L. Targeting the Dynamic HSP90 Complex in Cancer. Nat. Rev. Cancer 2010, 10, 537–549.
36. Zong, H.; Gozman, A.; Caldas-Lopes, E.; Taldone, T.; Sturgill, E.; Brennan, S.; Ochiana, S.O.; Gomes-DaGama, E.M.; Sen, S.; Rodina, A.; et al. A Hyperactive Signalosome in Acute Myeloid Leukemia Drives Addiction to a Tumor-Specific Hsp90 Species. Cell Rep. 2015, 13, 2159–2173.
37. Vander Heiden, M.G.; Cantley, L.C.; Thompson, C.B. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. Science 2009, 324, 1029–1033.
38. Martínez-Reyes, I.; Chandel, N.S. Mitochondrial TCA Cycle Metabolites Control Physiology and Disease. Nat. Commun. 2020, 11, 102.
39. Woodford, M.R.; Baker-Williams, A.J.; Sager, R.A.; Backe, S.J.; Blanden, A.R.; Hashmi, F.; Kancherla, P.; Gori, A.; Loiselle, D.R.; Castelli, M.; et al. The Tumor Suppressor Folliculin Inhibits Lactate Dehydrogenase A and Regulates the Warburg Effect. Nat. Struct. Mol. Biol. 2021, 28, 662–670.
40. DeBerardinis, R.J.; Chandel, N.S. We Need to Talk about the Warburg Effect. Nat. Metab. 2020, 2, 127–129.
41. Warburg, O. The Metabolism of Carcinoma Cells. J. Cancer Res. 1925, 9, 148–163.
42. Sanchez-Martin, C.; Moroni, E.; Ferraro, M.; Laquatra, C.; Cannino, G.; Masgras, I.; Negro, A.; Quadrelli, P.; Rasola, A.; Colombo, G. Rational Design of Allosteric and Selective Inhibitors of the Molecular Chaperone TRAP1. Cell Rep. 2020, 31, 107531.
43. Sanchez-Martin, C.; Menon, D.; Moroni, E.; Ferraro, M.; Masgras, I.; Elsey, J.; Arbiser, J.L.; Colombo, G.; Rasola, A. Honokiol Bis-Dichloroacetate Is a Selective Allosteric Inhibitor of the Mitochondrial Chaperone TRAP1. Antioxid. Redox Signal. 2021, 34, 505–516.
44. Sciacovelli, M.; Guzzo, G.; Morello, V.; Frezza, C.; Zheng, L.; Nannini, N.; Calabrese, F.; Laudiero, G.; Esposito, F.; Landriscina, M.; et al. The Mitochondrial Chaperone TRAP1 Promotes Neoplastic Growth by Inhibiting Succinate Dehydrogenase. Cell Metab. 2013, 17, 988–999.
45. Serapian, S.A.; Moroni, E.; Ferraro, M.; Colombo, G. Atomistic Simulations of the Mechanisms of the Poorly Catalytic Mitochondrial Chaperone Trap1: Insights into the Effects of Structural Asymmetry on Reactivity. ACS Catal. 2021, 11, 8605–8620.
46. Xiang, F.; Ma, S.; Lv, Y.; Zhang, D.; Song, H.; Huang, Y. Tumor Necrosis Factor Receptor-Associated Protein 1 Regulates Hypoxia-Induced Apoptosis through a Mitochondria-Dependent Pathway Mediated by Cytochrome c Oxidase Subunit II. Burn. Trauma 2019, 7, s41038-019-0154-3s41038–s019.

47. Xiang, F.; Ma, S.-Y.; Zhang, D.-X.; Zhang, Q.; Huang, Y.-S. Tumor Necrosis Factor Receptor-Associated Protein 1 Improves Hypoxia-Impaired Energy Production in Cardiomyocytes through Increasing Activity of Cytochrome c Oxidase Subunit II. Int. J. Biochem. Cell Biol. 2016, 79, 239–248.

48. Bezawork-Geleta, A.; Rohlena, J.; Dong, L.; Pacak, K.; Neuzil, J. Mitochondrial Complex II: At the Crossroads. Trends Biochem. Sci. 2017, 42, 312–325.

49. Liu, Y.; Elnatan, D.; Sun, M.; Myasnikov, A.G.; Agard, D.A. Cryo-EM Reveals the Dynamic Interplay between Mitochondrial Hsp90 and SdhB Folding Intermediates. bioRxiv 2020.

50. Agarwal, E.; Altman, B.J.; Seo, J.H.; Ghosh, J.C.; Kossenkov, A.V.; Tang, H.-Y.; Krishn, S.R.; Languino, L.R.; Gabrilovich, D.I.; Speicher, D.W.; et al. Myc-Mediated Transcriptional Regulation of the Mitochondrial Chaperone TRAP1 Controls Primary and Metastatic Tumor Growth. J. Biol. Chem. 2019, 294, 10407–10414.

51. Chae, Y.C.; Angelin, A.; Lisanti, S.; Kossenkov, A.V.; Speicher, K.D.; Wang, H.; Powers, J.F.; Tischler, A.S.; Pacak, K.; Fliedner, S.; et al. Landscape of the Mitochondrial Hsp90 Metabolome in Tumours. Nat. Commun. 2013, 4, 2139.

52. Hu, S.; Ferraro, M.; Thomas, A.P.; Chung, J.M.; Yoon, N.G.; Seol, J.-H.; Kim, S.; Kim, H.; An, M.Y.; Ok, H.; et al. Dual Binding to Orthosteric and Allosteric Sites Enhances the Anticancer Activity of a TRAP1-Targeting Drug. J. Med. Chem. 2020, 63, 2930–2940.

53. Masgras, I.; Sanchez-Martin, C.; Colombo, G.; Rasola, A. The Chaperone TRAP1 As a Modulator of the Mitochondrial Adaptations in Cancer Cells. Front. Oncol. 2017, 7, 58.

54. Rizza, S.; Montagna, C.; Cardaci, S.; Maiani, E.; Di Giacomo, G.; Sanchez-Quiles, V.; Blagoev, B.; Rasola, A.; De Zio, D.; Stamler, J.S.; et al. S-Nitrosylation of the Mitochondrial Chaperone TRAP1 Sensitizes Hepatocellular Carcinoma Cells to Inhibitors of Succinate Dehydrogenase. Cancer Res. 2016, 76, 4170–4182.

55. Fitzgerald, J.C.; Zimprich, A.; Carvajal Berrio, D.A.; Schindler, K.M.; Maurer, B.; Schulte, C.; Bus, C.; Hauser, A.-K.; Kübler, M.; Lewin, R.; et al. Metformin Reverses TRAP1 Mutation-Associated Alterations in Mitochondrial Function in Parkinson’s Disease. Brain 2017, 140, 2444–2459.

56. Guzzo, G.; Sciacovelli, M.; Bernardi, P.; Rasola, A. Inhibition of Succinate Dehydrogenase by the Mitochondrial Chaperone TRAP1 Has Anti-Oxidant and Anti-Apoptotic Effects on Tumor Cells. Oncotarget 2014, 5, 11897–11908.

57. Hsu, C.-C.; Tseng, L.-M.; Lee, H.-C. Role of Mitochondrial Dysfunction in Cancer Progression. Exp. Biol. Med. 2016, 241, 1281–1295.

58. Selak, M.A.; Armour, S.M.; MacKenzie, E.D.; Boulahbel, H.; Watson, D.G.; Mansfield, K.D.; Pan, Y.; Simon, M.C.; Thompson, C.B.; Gottlieb, E. Succinate Links TCA Cycle Dysfunction to Oncogenesis by Inhibiting HIF-α Prolyl Hydroxylase. Cancer Cell 2005, 7, 77–85.

59. Zong, S.; Wu, M.; Gu, J.; Liu, T.; Guo, R.; Yang, M. Structure of the Intact 14-Subunit Human Cytochrome c Oxidase. Cell Res. 2018, 28, 1026–1034.
60. Lisanti, S.; Tavecchio, M.; Chae, Y.C.; Liu, Q.; Brice, A.K.; Thakur, M.L.; Languino, L.R.; Altieri, D.C. Deletion of the Mitochondrial Chaperone TRAP-1 Uncovers Global Reprogramming of Metabolic Networks. Cell Rep. 2014, 8, 671–677.
61. Sullivan, L.B.; Chandel, N.S. Mitochondrial Reactive Oxygen Species and Cancer. Cancer Metab. 2014, 2, 17.
62. Clarke, B.E.; Kalmar, B.; Greensmith, L. Enhanced Expression of TRAP1 Protects Mitochondrial Function in Motor Neurons under Conditions of Oxidative Stress. Int. J. Mol. Sci. 2022, 23, 1789.
63. Kang, B.H.; Plescia, J.; Dohi, T.; Rosa, J.; Doxsey, S.J.; Altieri, D.C. Regulation of Tumor Cell Mitochondrial Homeostasis by an Organelle-Specific Hsp90 Chaperone Network. Cell 2007, 131, 257–270.
64. Leav, I.; Plescia, J.; Goel, H.L.; Li, J.; Jiang, Z.; Cohen, R.J.; Languino, L.R.; Altieri, D.C. Cytoprotective Mitochondrial Chaperone TRAP-1 As a Novel Molecular Target in Localized and Metastatic Prostate Cancer. Am. J. Pathol. 2010, 176, 393–401.
65. Vartholomaiou, E.; Madon-Simon, M.; Hagmann, S.; Mühlebach, G.; Wurst, W.; Floss, T.; Picard, D. Cytosolic Hsp90α and Its Mitochondrial Isoform Trap1 Are Differentially Required in a Breast Cancer Model. Oncotarget 2017, 8, 17428–17442.
66. Linehan, W.M.; Schmidt, L.S.; Crooks, D.R.; Wei, D.; Srinivasan, R.; Lang, M.; Ricketts, C.J. The Metabolic Basis of Kidney Cancer. Cancer Discov. 2019, 9, 1006–1021.
67. Semenza, G.L. HIF-1: Upstream and Downstream of Cancer Metabolism. Curr. Opin. Genet. Dev. 2010, 20, 51–56.
68. Kowalik, M.A.; Guzzo, G.; Morandi, A.; Perra, A.; Menegon, S.; Masgras, I.; Trevisan, E.; Angioni, M.M.; Fornari, F.; Quagliata, L.; et al. Metabolic Reprogramming Identifies the Most Aggressive Lesions at Early Phases of Hepatic Carcinogenesis. Oncotarget 2016, 7, 32375–32393.
69. Iacobazzi, V.; Infantino, V. Citrate—New Functions for an Old Metabolite. Biol. Chem. 2014, 395, 387–399.
70. Liu, J.; Peng, Y.; Wei, W. Cell Cycle on the Crossroad of Tumorigenesis and Cancer Therapy. Trends Cell Biol. 2022, 32, 30–44.
71. Amoroso, M.R.; Matassa, D.S.; Laudiero, G.; Egorova, A.V.; Polishchuk, R.S.; Maddalena, F.; Piscazzi, A.; Paladino, S.; Sarnataro, D.; Garbi, C.; et al. TRAP1 and the Proteasome Regulatory Particle TBP7/Rpt3 Interact in the Endoplasmic Reticulum and Control Cellular Ubiquitination of Specific Mitochondrial Proteins. Cell Death Differ. 2012, 19, 592–604.

72. Sisinni, L.; Maddalena, F.; Condelli, V.; Pannone, G.; Simeon, V.; Li Bergolis, V.; Lopes, E.; Piscazzi, A.; Matassa, D.S.; Mazzoccoli, C.; et al. TRAP1 Controls Cell Cycle G2-M Transition through the Regulation of CDK1 and MAD2 Expression/Ubiquitination: TRAP1 Regulates Mitotic Entry through CDK1 Quality Control. J. Pathol. 2017, 243, 123–134.

73. Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. Induction of Apoptotic Program in Cell-Free Extracts: Requirement for DATP and Cytochrome c. Cell 1996, 86, 147–157.

74. Li, P.; Nijhawan, D.; Budihardjo, I.; Srinivasula, S.M.; Ahmad, M.; Alnemri, E.S.; Wang, X. Cytochrome c and DATP-Dependent Formation of Apaf-1/Caspase-9 Complex Initiates an Apoptotic Protease Cascade. Cell 1997, 91, 479–489.

75. Javadov, S.; Chapa-Dubocq, X.; Makarov, V. Different Approaches to Modeling Analysis of Mitochondrial Swelling. Mitochondrion 2018, 38, 58–70.

76. Orrenius, S.; Gogvadze, V.; Zhivotovsky, B. Calcium and Mitochondria in the Regulation of Cell Death. Biochem. Biophys. Res. Commun. 2015, 460, 72–81.

77. Bernardi, P.; Rasola, A.; Forte, M.; Lippe, G. The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology. Physiol. Rev. 2015, 95, 1111–1155.

78. Redza-Dutordoir, M.; Averill-Bates, D.A. Activation of Apoptosis Signalling Pathways by Reactive Oxygen Species. Biochim. Et Biophys. Acta (BBA)-Mol. Cell Res. 2016, 1863, 2977–2992.

79. Hua, G.; Zhang, Q.; Fan, Z. Heat Shock Protein 75 (TRAP1) Antagonizes Reactive Oxygen Species Generation and Protects Cells from Granzyme M-Mediated Apoptosis. J. Biol. Chem. 2007, 282, 20553–20560.

80. Im, C.-N.; Lee, J.-S.; Zheng, Y.; Seo, J.-S. Iron Chelation Study in a Normal Human Hepatocyte Cell Line Suggests That Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP1) Regulates Production of Reactive Oxygen Species. J. Cell. Biochem. 2007, 100, 474–486.

81. Costantino, E.; Maddalena, F.; Calise, S.; Piscazzi, A.; Tirino, V.; Fersini, A.; Ambrosi, A.; Neri, V.; Esposito, F.; Landriscina, M. TRAP1, a Novel Mitochondrial Chaperone Responsible for Multi-Drug Resistance and Protection from Apoptotis in Human Colorectal Carcinoma Cells. Cancer Lett. 2009, 279, 39–46.

82. Landriscina, M.; Laudiero, G.; Maddalena, F.; Amoroso, M.R.; Piscazzi, A.; Cozzolino, F.; Monti, M.; Garbi, C.; Fersini, A.; Pucci, P.; et al. Mitochondrial Chaperone Trap1 and the Calcium Binding Protein Sorcin Interact and Protect Cells against Apoptosis Induced by Antiblastic Agents. Cancer Res. 2010, 70, 6577–6586.

83. Suarez, J.; McDonough, P.M.; Scott, B.T.; Suarez-Ramirez, A.; Wang, H.; Fricovsky, E.S.; Dillmann, W.H. Sorcin Modulates Mitochondrial Ca2+ Handling and Reduces Apoptosis in Neonatal Rat Cardiac Myocytes. Am. J. Physiol. -Cell Physiol. 2013, 304, C248–C256.

84. Elnatan, D.; Agard, D.A. Calcium Binding to a Remote Site Can Replace Magnesium as Cofactor for Mitochondrial Hsp90 (TRAP1) ATPase Activity. J. Biol. Chem. 2018, 293, 13717–13724.
85. Zhang, L.; Su, N.; Luo, Y.; Chen, S.; Zhao, T. TRAP1 Inhibits MIC60 Ubiquitination to Mitigate the Injury of Cardiomyocytes and Protect Mitochondria in Extracellular Acidosis. Cell Death Discov. 2021, 7, 389.
86. Friedman, J.R.; Mourier, A.; Yamada, J.; McCaffery, J.M.; Nunnari, J. MICOS Coordinates with Respiratory Complexes and Lipids to Establish Mitochondrial Inner Membrane Architecture. eLife 2015, 4, e07739.
87. Rampelt, H.; Zerbes, R.M.; van der Laan, M.; Pfanner, N. Role of the Mitochondrial Contact Site and Cristae Organizing System in Membrane Architecture and Dynamics. Biochim. Et Biophys. Acta (BBA)-Mol. Cell Res. 2017, 1864, 737–746.
88. Baines, C.P.; Gutiérrez-Aguilar, M. The Mitochondrial Permeability Transition Pore: Is It Formed by the ATP Synthase, Adenine Nucleotide Translocators or Both? Biochim. Et Biophys. Acta (BBA)-Bioenerg. 2020, 1861, 148249.
89. Carrer, A.; Tommasin, L.; Šileikytė, J.; Ciscato, F.; Filadi, R.; Urbani, A.; Forte, M.; Rasola, A.; Szabò, I.; Carraro, M.; et al. Defining the Molecular Mechanisms of the Mitochondrial Permeability Transition through Genetic Manipulation of F-ATP Synthase. Nat. Commun. 2021, 12, 4835.
90. Giorgio, V.; von Stockum, S.; Antoniel, M.; Fabbro, A.; Fogolari, F.; Forte, M.; Glick, G.D.; Petronilli, V.; Zoratti, M.; Szabó, I.; et al. Dimers of Mitochondrial ATP Synthase Form the Permeability Transition Pore. Proc. Natl. Acad. Sci. USA 2013, 110, 5887–5892.
91. Kang, B.H.; Tavecchio, M.; Goel, H.L.; Hsieh, C.-C.; Garlick, D.S.; Raskett, C.M.; Lian, J.B.; Stein, G.S.; Languino, L.R.; Altieri, D.C. Targeted Inhibition of Mitochondrial Hsp90 Suppresses Localised and Metastatic Prostate Cancer Growth in a Genetic Mouse Model of Disease. Br. J. Cancer 2011, 104, 629–634.
92. Baines, C.P.; Kaiser, R.A.; Purcell, N.H.; Blair, N.S.; Osinska, H.; Hambleton, M.A.; Brunskill, E.W.; Sayen, M.R.; Gottlieb, R.A.; Dorn, G.W.; et al. Loss of Cyclophilin D Reveals a Critical Role for Mitochondrial Permeability Transition in Cell Death. Nature 2005, 434, 658–662.
93. Matassa, D.S.; Amoroso, M.R.; Maddalena, F.; Landriscina, M. New Insights into TRAP1 Pathway. Am. J. Cancer Res. 2012, 2, 235–248.
94. Ghosh, J.C.; Siegelin, M.D.; Dohi, T.; Altieri, D.C. Heat Shock Protein 60 Regulation of the Mitochondrial Permeability Transition Pore in Tumor Cells. Cancer Res. 2010, 70, 8988–8993.
95. Lebedev, I.; Nemajerova, A.; Foda, Z.H.; Kornaj, M.; Tong, M.; Moll, U.M.; Seeliger, M.A. A Novel In Vitro CypD-Mediated P53 Aggregation Assay Suggests a Model for Mitochondrial Permeability Transition by Chaperone Systems. J. Mol. Biol. 2016, 428, 4154–4167.

96. Sinha, D.; D'Silva, P. Chaperoning Mitochondrial Permeability Transition: Regulation of Transition Pore Complex by a J-Protein, DnaJC15. Cell Death Dis. 2014, 5, e1101.
97. Niemi, N.M.; Pagliarini, D.J. The Extensive and Functionally Uncharacterized Mitochondrial Phosphoproteome. J. Biol. Chem. 2021, 297, 100880.
98. Faienza, F.; Lambrughi, M.; Rizza, S.; Pecorari, C.; Giglio, P.; Salamanca Viloria, J.; Allega, M.F.; Chiappetta, G.; Vinh, J.; Pacello, F.; et al. S-Nitrosylation Affects TRAP1 Structure and ATPase Activity and Modulates Cell Response to Apoptotic Stimuli. Biochem. Pharmacol. 2020, 176, 113869.
99. Rasola, A.; Neckers, L.; Picard, D. Mitochondrial Oxidative Phosphorylation TRAP(1)Ped in Tumor Cells. Trends Cell Biol. 2014, 24, 455–463.
100. Cloutier, P.; Coulombe, B. Regulation of Molecular Chaperones through Post-Translational Modifications: Decrypting the Chaperone Code. Biochim. Biophys. Acta (BBA)-Gene Regul. Mech. 2013, 1829, 443–454.
101. Zuehlke, A.D.; Reidy, M.; Lin, C.; LaPointe, P.; Alsomairy, S.; Lee, D.J.; Rivera-Marquez, G.M.; Beebe, K.; Prince, T.; Lee, S.; et al. An Hsp90 Co-Chaperone Protein in Yeast Is Functionally Replaced by Site-Specific Posttranslational Modification in Humans. Nat. Commun. 2017, 8, 15328.