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The molecular chaperone TRAP1 in cancer: From the basics of biology to pharmacological targeting

Ionica Masgras [a,b], Claudio Laquatra [a], Giuseppe Cannino [a], Stefano A. Serapian [c], Giorgio Colombo [c,**], Andrea Rasola [a,*]

[a] Dipartimento di Scienze Biomediche, Università di Padova, Padova, Italy
[b] Istituto di Neuroscienze, Consiglio Nazionale Delle Ricerche (CNR), Padova, Italy
[c] Dipartimento di Chimica, Università di Pavia, Pavia, Italy

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ABSTRACT

TRAP1, the mitochondrial component of the Hsp90 family of molecular chaperones, displays important bioenergetic and proteostatic functions. In tumor cells, TRAP1 contributes to shape metabolism, dynamically tuning it with the changing environmental conditions, and to shield from noxious insults. Hence, TRAP1 activity has profound effects on the capability of neoplastic cells to evolve towards more malignant phenotypes. Here, we discuss our knowledge on the biochemical functions of TRAP1 in the context of a growing tumor mass, and we analyze the possibility of targeting its chaperone functions for developing novel anti-neoplastic approaches.

1. The mitochondrial chaperone TRAP1: the basics

TRAP1, also called Hsp75, is the mitochondrial paralog of the Hsp90 molecular chaperone, with which it shares domain organization and a high degree of amino acid sequence homology. Hsp90 family chaperones are molecular machines that control the structure and activity of a variety of diverse proteins, termed clients, by allowing them to undergo conformational changes, to reach specific subcellular localizations or to form multimeric complexes, and by regulating their degradation following aggregation, unfolding or misfolding. The biochemical outputs of these functions consist in the integration of signaling and metabolic circuits, ultimately leading to a global proteostasis that can be seen as a dynamic process of quality control and efficiency maintenance of the proteome under changing environmental conditions [1].

In the case of TRAP1, its mitochondrial localization makes it a suitable candidate for the tuning of metabolic processes that take place in these organelles, and for responding to noxious conditions that affect mitochondrial physiology. Indeed, several observations indicate that TRAP1 expression or activity are increased under pathological conditions. In ischemic brain injury, TRAP1 induction shields from oxidative stress and tissue damage [2]. TRAP1 exerts a similar protective effect on hypoxic cardiomyocytes [3], in hearts exposed to ischemia/reperfusion, where it decreases ROS generation and maintains mitochondrial bioenergetic functions [4], and by preventing cardiac hypertrophy after aortic banding in mouse models [5]. Several lines of evidence also indicate that TRAP1 has a protective role in genetic models of Parkinson's disease (PD) [6–8], a condition where a poor quality control of mitochondria plays key pathogenic roles [9], and a loss of function TRAP1 mutation was found in a PD patient [10]. TRAP1 mutations have been retrieved also in a case of severe autoinflammation accompanied by redox disequilibrium in cells [11], in a child with the mitochondrial disease Leigh syndrome [12] and associated with chronic pain, fatigue and gastrointestinal dysmotility [13] and in a small fraction of patients with complex developmental syndromes characterized by malformations in the kidney and other districts [14]. Accordingly, it was proposed that TRAP1 protects mitochondria from damage in models of kidney fibrosis [15,16], and a TRAP1 mutation was observed in a proband with renal and thyroid cancers [17]. Most information on a possible pathological role of TRAP1 comes from tumor models, as the chaperone is highly expressed in patient cohorts with hepatocellular carcinoma (HCC) [18], small cell lung cancer [19], high-grade glioma [20], breast cancer [21], and ovarian, kidney, prostate, esophageal and colorectal cancer, where it is associated with advanced stage, metastasis and poor prognosis [22–28]. In colorectal cancer associated with ulcerative colitis and in an animal model of HCC progression, TRAP1 induction precedes the neoplastic changes and is restricted to lesions that progress to cancer

* Corresponding author at: Dipartimento di Scienze Biomediche, Università di Padova, viale G. Colombo 3, 35131, Padova, Italy.
** Corresponding author at: Dipartimento di Chimica, Università di Pavia, via Taramelli 12, 27100, Pavia, Italy.
E-mail addresses: g.colombo@unipv.it (G. Colombo), andrea.rasola@unipd.it (A. Rasola).

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[29,30]. These observations suggest an important role of TRAP1 in the metabolic adaptations that sustain neoplastic progression, and have prompted research aimed at mechanistically elucidate its biochemical activities in several cancer settings. In recent years, details on its structure, mode of action, interactors and functional effects have emerged, as well as the identification of selective inhibitors. These data are starting to compose a picture that identifies TRAP1 as a master switch in tumor bioenergetics.

2. Molecular structure of TRAP1 and regulation of its chaperone activity

The TRAP1 gene is evolutionary conserved, as it is present in mammals, insects, nematodes and even in the slime mold *Dictyostelium discoideum*, where it is involved in the response to starvation [31]. All TRAP1 sequences have an amino terminal mitochondrial targeting sequence [32]. The human TRAP1 locus is on chromosome 16p13 and the mature protein is composed by a N-terminal regulatory domain (NTD) required for ATP binding, a middle domain (MD) responsible for ATP hydrolysis and client binding, and a C-terminal domain (CTD) involved in the dimerization of two TRAP1 protomers [33,34]. Dimers of TRAP1 utilize ATP to undergo a chaperone cycle composed of a series of conformational changes [35]. ATP binding promotes a shift from an "apo" (open) state to a closed conformation that is asymmetric due to the buckling of one protomer (Fig. 1) [36]. Dimer closure is the rate-limiting step of the process [37] and it prompts ATP hydrolysis in a sequential way. After the first ATPase event, which occurs in the buckled protomer, the dimer asymmetry flips so that the remaining ATP-bound protomer bents and undergoes the second ATP hydrolysis [38]. The first reaction would couple client remodeling, whereas the second would lead to its dissociation and to the return of TRAP1 to the apo state [39]. The MD: CTD interface is critical for client binding, and it is believed that a variety of distinct open states can bind a diverse set of clients. A structural crosstalk between the NTD and the distal site of client binding dynamically couples client recognition with ATP hydrolysis, supporting the hypothesis that structural asymmetry plays a role in regulating TRAP1 chaperone properties [40]. Notably, TRAP1 activity displays a marked temperature sensitivity, with a 200-fold increase of ATPase activity between 25 °C and 55 °C [37], that depends on a N-terminal extension termed "strap" that stabilizes the closed state through *trans*-protomer interactions [39]. The same strap is instead bound to the *cis* protomer in the open state [41]. This increased TRAP1 activity at higher temperatures is intriguing, as it could have an important proteostatic function in mitochondria that are warmer than the rest of the cell [42]. The TRAP1 ATPase pocket can also bind Ca^{2+} instead of Mg^{2+} as enzymatic cofactor. When bound to Ca^{2+}, TRAP1 switches its hydrolytic activity from a sequential to a cooperative mode, thus skipping the asymmetry flipping and the ensuing client remodeling that characterize its normal chaperone cycle. Under these conditions, TRAP1 directly moves from closure to full opening, and TRAP1 ATPase activity is higher with Ca^{2+} at high ATP concentrations [38]. This short-circuited cycle could be consistent with a holdase behaviour of TRAP1, in which a capture and

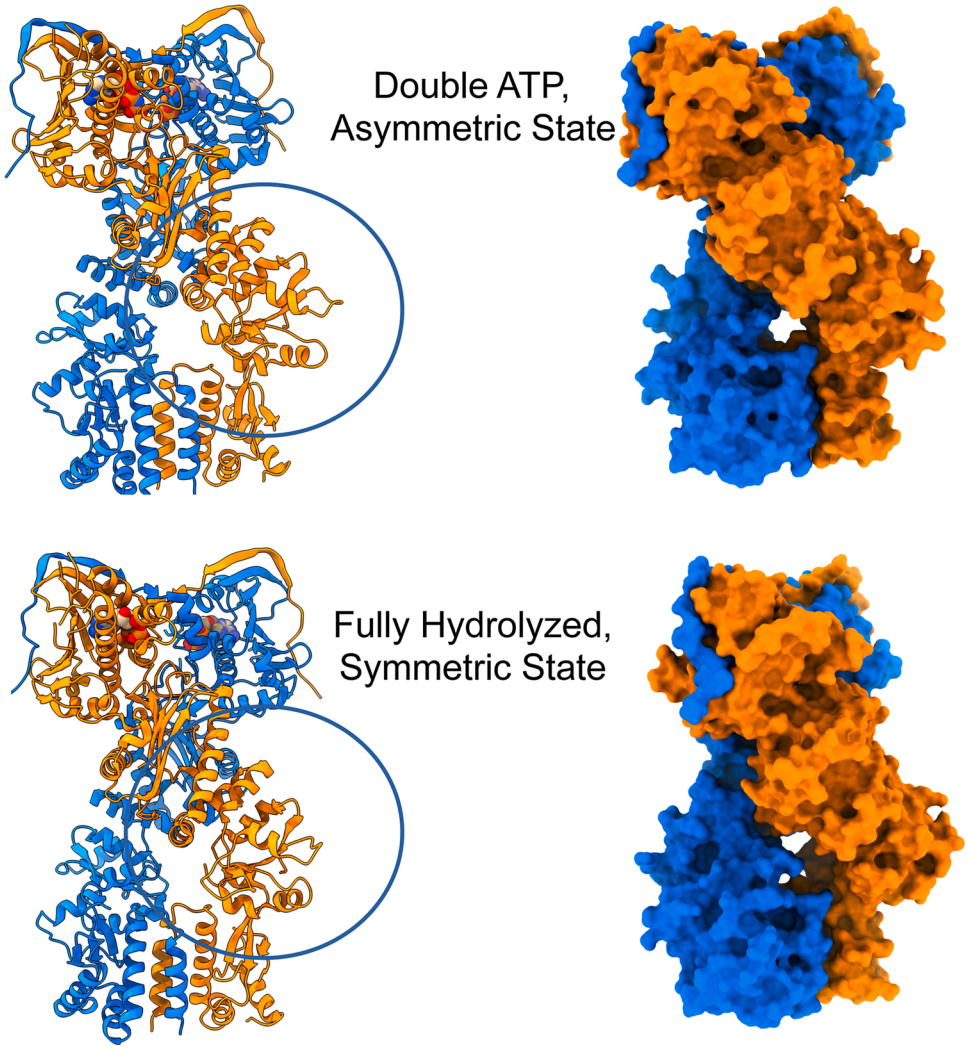

Fig. 1. The three dimensional structures of TRAP1 in different nucleotide states. The two different protomers are colored in orange and blue, respectively, and are shown both as secondary structures and as surfaces. The circle highlights the substructure in the middle domain that coincides with the client binding site and undergoes rearrangement upon nucleotide hydrolysis. This substructure is the target for the design of allosteric inhibitors. Top row: the peculiar asymmetric state of TRAP1 in the double ATP state (taken from PDB structure code: 2ipe.pdb). Bottom row: the symmetric state of TRAP1 in the fully hydrolyzed state of the protein (taken from PDB structure code: 5twx.pdb).

release mechanism would avoid protein aggregations under stress conditions. Therefore, the choice of the divalent cation could confer flexibility to TRAP1 activity, possibly determining distinct subset of clients in response to the local changes of Ca^{2+} concentration that frequently occur in mitochondrial microdomains.

Recent cryo-EM investigations indicate that TRAP1 can also form tetramers with parallel, antiparallel or orthogonal conformations. This last configuration, termed "butterfly" tetramer, was determined at 3.5 Å resolution with each dimer in the closed state, finding three dimer-dimer interaction regions. In this model, the CTD can open and close in both dimeric and tetrameric TRAP1 when the chaperone is closed and bound to ATP, and TRAP1 could switch between different tetrameric conformations by CTD domain swapping [43]. The functional meaning of these higher order conformations remains unknown, but their identification opens the fascinating scenario of a further plasticity in TRAP1 functions. For instance, TRAP1 tetramers could be suited for chaperoning large multiprotein complexes, such as those that constitute the oxidative phosphorylation (OXPHOS) machinery. Accordingly, the presence of TRAP1 tetramers is enhanced following perturbations in OXPHOS activity [44]. Cryo-EM analyses also allowed to resolve the first structure of a complex between TRAP1 and a client, the subunit B of the respiratory complex II enzyme succinate dehydrogenase (SDH). SDH oxidizes succinate to fumarate and funnels electrons to the respiratory complex III. Hence, it is positioned at the crossroad of OXPHOS machinery and tricarboxylic acid (TCA) cycle, which confers crucial bioenergetic effects to SDH with a particular relevance in tumors. It is indeed well-known [45] that TRAP1 down-regulates SDH, causing accumulation of its substrate succinate that acts as a competitive inhibitor of α-ketoglutarate-dependent dioxygenases, leading to an array of pro-neoplastic effects (see below) [35]. Cryo-EM inspections [46] could determine that the TRAP1/SDHB interaction accelerates TRAP1 dimer closure and ATP hydrolysis and elicits an unexpected transition to symmetry of the closed TRAP1 dimer (Fig. 1). The Authors hypothesize that TRAP1 could bind to a SDHB polypeptide just translocated into mitochondria, thus taking part to the process of protein import, and that the chaperone could contribute to the loading of the iron-sulfur cluster during SDHB folding. These functions of TRAP1 would extend beyond its interaction with SDH, providing mechanistic hints for a more general control by TRAP1 of bioenergetic activity and of proteostasis in mitochondria. Therefore, the growing comprehension of the structural details of TRAP1 biochemistry is instrumental to better dissect and target its functions in the neoplastic process.

3. TRAP1 in cancer: an expanding network of effects

Cancer cells experience a variety of stresses that reverberate in mitochondrial changes aimed at maintaining homeostasis and functional fitness. Fluctuating nutrient and oxygen availability from the microenvironment cause profound rewiring of metabolic circuities and unbalances in redox equilibrium [47], potentially hampering protein conformation and functionality and eliciting genomic instability. This results in the risk of amplifying damage to mitochondrial structure and function in cells kept under the pressure of unrelenting proliferation [48,49]. In this context, TRAP1 can play an important proteostatic role acting in several ways to finely adjust bioenergetic and survival pathways to the changing cell requirements.

3.1. TRAP1 as a gatekeeper in tumor cell survival

It is established that TRAP1 confers resistance to oxidative stress following activation of a NK cell cytolytic program involving Granzyme M [50] and in several tumor cell models [33,51,52]. This anti-oxidant activity of TRAP1 protects tumor cells from apoptosis [21,53–55] and increases their chemoresistance and their invasive and metastatic properties [26,56,57]. The first mechanistic clues to explain these pro-neoplastic functions of TRAP1 came when it was shown that it interacts with cyclophilin D (CyPD) [58], a peptidyl-prolyl isomerase that sensitizes to opening the permeability transition pore (PTP). The PTP is a channel that opens following ROS or Ca^{2+} surges inside mitochondria, rapidly eliciting cell death [59,60], and its inhibition could constitute a strategy adopted by tumor cells to escape the lethal effects of noxious stimuli [61]. TRAP1 inhibition of PTP opening and of the ensuing apoptosis was observed not only in tumor cells [33,51], but also in models of neural stem cells [62], in cardiomyocytes exposed to an acidic milieu [63] and in kidney cells under high glucose conditions prompting oxidative stress and mimicking the diabetic kidney disease [64], thus highlighting the general pathophysiological relevance of this pro-survival TRAP1 activity. An interesting model proposes that the CyPD/TRAP1 interaction can be displaced by p53 entering mitochondria under oxidative stress. As a result, CyPD would activate its isomerase function, leading to p53 aggregation into amyloid-like fibrils and to structural changes in PTP components, ultimately causing PTP opening and cell death [65]. As oxidative stress can favor genetic instability and neoplastic progression of transformed cells, this mechanism adds to the disparate anti-neoplastic activities of p53 [66], and specularly places TRAP1 in a key position to maintain tumor cell viability. Recent evidences indicating that F-ATP synthase forms the PTP [67–69] offer fresh perspectives to elucidate in greater detail the molecular mechanisms by which TRAP1 tunes PTP opening, possibly affecting conformations of specific subunits of the F-ATP synthase holoenzyme required for channel formation. Accordingly, a direct interaction between TRAP1 and F-ATP synthase components has been reported [44].

3.2. TRAP1 in the complexity of the neoplastic process(es)

The ROS buffering effect exerted by TRAP1 could also explain contrasting reports on its role in the tumorigenic process. Indeed, it was observed that TRAP1 inversely correlates with tumor grade and stage in specific settings of renal cell carcinoma and ovarian cancer [70,71], and its expression only delays the appearance of tumors in a mouse model of mammary carcinogenesis, without affecting tumor burden or metastases [72]. To explain these context-dependent effects of TRAP1 on the growth of malignant cells, it can be useful to think of the tumor mass as a structure evolving under changing environmental conditions, where intracellular redox equilibria follow unstable patterns. In the early phases of tumorigenesis, exposing cells to high surges of ROS could prompt PTP opening and cell death, thus inhibiting neoplastic progression. On the contrary, a higher degree of oxidative stress could be advantageous for malignant cells by boosting their genetic instability and favoring clonal diversity, leading to a higher capability to respond to chemotherapeutics or to settle in metastatic niches (discussed in [34]). Further layers of complexity when dissecting TRAP1 functions in tumors could be provided by its interactions with multiple clients, including other chaperones, metabolic enzymes and transporters [44]. As a consequence, TRAP1 could modulate proteostatic responses and tune bioenergetic circuits in modes that are differentially shaped by the mitochondrial proteome activity in different tumor types. TRAP1 could also intervene in the metabolic crosstalk between neoplastic and microenvironment cells, a fascinating possibility that deserves a thorough investigation. Moreover, specific subsets of cells in the neoplastic mass could display different metabolic features, at least partially determined by TRAP1. For instance, TRAP1 was found co-expressed with stem cell markers [73], and in glioma stem cells (GSCs) TRAP1 contributes to maintain stemness by sustaining their metabolic plasticity [74]. Hence, TRAP1 could be instrumental to allow adaptations to conditions of nutrient and oxygen paucity that frequently characterize the stem cell compartment. Accordingly, TRAP1 is highly induced in patients with malignant forms of glioma, and its inactivation in GSCs suppresses tumor onset in *in vivo* glioma models [74].

In the last years, research has tried to put TRAP1 in this scenario of tumor complexity (Fig. 2). Novel interactors and regulatory mechanisms

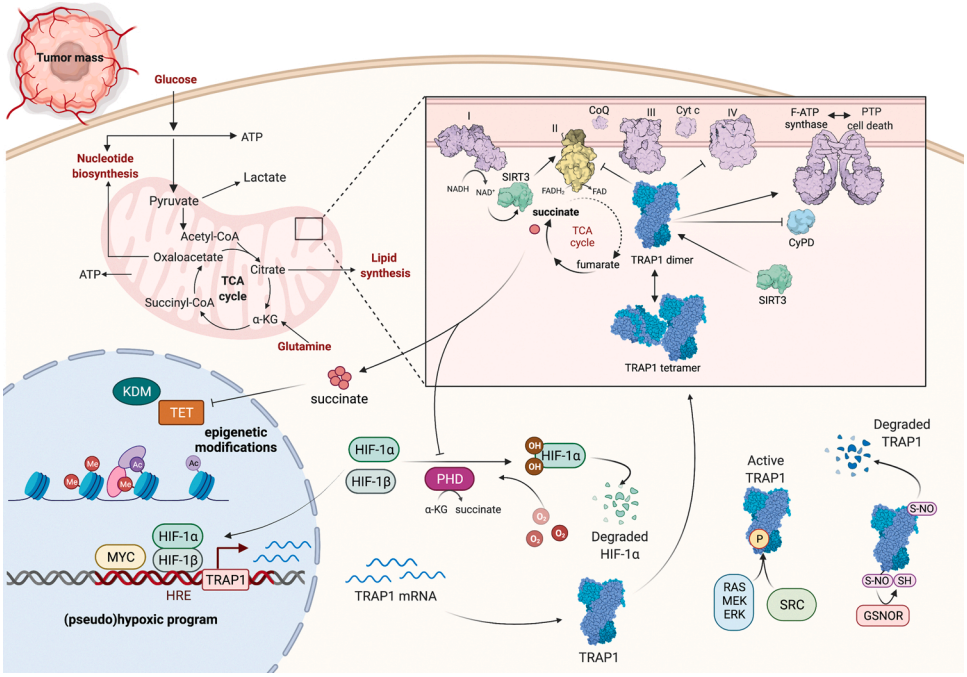

Fig. 2. TRAP1 regulatory pathways in the tumor cell. In mitochondria, TRAP1 down-regulates the activity of OXPHOS complexes II and IV (SDH and cytochrome *c* oxidase, respectively) and it interacts with CyPD and F-ATP synthase, inhibiting PTP opening, and with the NAD^+-dependent deacetylase SIRT3. The chaperone can flip between a dimer and a tetramer conformation, with unknown effects. TRAP1 activity is increased by phosphorylations, downstream to tyrosine kinase Src and Ras/MEK/ERK signalling, whereas its S-nitrosylation primes it for degradation. Succinate accumulation following TRAP1-dependent SDH inhibition elicits epigenetic changes and HIF1α stabilization that activates a pseudo-hypoxic program. In turn, HIF1α as well as Myc trigger the transcription of the *TRAP1* gene.

of TRAP1 expression and function are emerging, thus delineating the possibility that dynamic protein networks assemble under specific conditions of stress and are orchestrated by TRAP1 to allow tumor cells managing harmful environmental situations.

3.3. *Turning on and off TRAP1*

Fine regulation of TRAP1 activity can occur in several ways. In spite of a lack of knowledge on the promoter region of the *TRAP1* gene, its transcription was found induced in a Myc-dependent way, first in an *in silico* microarray analysis [75] and then in a model of neuroblastoma. In this cancer type, N-Myc elicits tumor progression and its inhibition induces mitochondrial damage in cancer cells by down-modulating TRAP1 and increasing ROS [52]. The crucial role of Myc hyperactivation in a plethora of tumor types [76], where it orchestrates diverse biological processes that include the response to metabolic stress conditions [77], suggests that TRAP1 could be a general effector of these pro-neoplastic bioenergetic changes.

TRAP1 up-regulation in T-cell acute lymphoblastic leukemia (T-ALL) was associated with the apoptosis resistance that characterizes this disease. In T-ALL, TRAP1 induction occurs downstream to inactivating mutations in components of the polycomb repressive complex 2 (PRC2). PRC2 inhibition activates the CRIP2 transcription factor and leads to the ensuing TRAP1 induction [78]. Loss of a functional PRC2 complex also enhances oncogenic Ras signalling [79] and characterizes progression to malignancy of tumors related to the genetic syndrome neurofibromatosis type 1 (NF1), where the Ras pathway is dysregulated by ablation of the Ras-GAP neurofibromin [80]. In NF1 tumor models, TRAP1 activity promotes neoplastic growth [81] and it is tempting to speculate that its expression can be enhanced *via* PRC2 inactivation.

The *TRAP1* promoter contains hypoxia responsive elements (HREs) that are targeted by the transcription factor HIF1 under hypoxic conditions, and TRAP1 expression is increased by HIF1 in hypoxia in human models of pancreatic cancer, glioblastoma and plexiform neurofibroma, as well as during Zebrafish embryonic development. Genetic or pharmacological inhibition of TRAP1 maintains a high level of respiration when oxygen is scarce [82], indicating that TRAP1 acts as a major OXPHOS repressor in these stressful conditions. HIF1 orchestrates a complex transcriptional program that shapes cell metabolism by inducing glycolysis and repressing OXPHOS, promotes proliferation and motility of cells and elicits angiogenesis, with a major impact on cancer cell malignancy [83].

Not only TRAP1 is a HIF1 effector, but it can also trigger its transcriptional activity *via* the raise in intracellular succinate that follows TRAP1-dependent down-regulation of SDH [45]. Succinate acts as an oncometabolite, *i.e.* it favors tumor growth, by competitively blocking the activity of α-ketoglutarate–dependent dioxygenases [84]. These include prolyl hydroxylases (PHDs), the inhibition of which stabilizes HIF1α and leads to the induction of the HIF1 transcriptional program independently of oxygen tension, the so-called pseudohypoxic phenotype that equips neoplastic cells to tackle the harsh conditions of growth in an irregularly vascularized milieu [85]. Therefore, TRAP1 is at the core of a feed-forward loop where it induces HIF1α, which in turn increases TRAP1 levels (Fig. 2). This crosstalk could play important roles when cells experience wobbly metabolic conditions, as it could couple cell bioenergetic status with environmental cues, such as changing nutrient and oxygen levels, in order to establish the most appropriate biological responses.

Succinate can also inhibit the JmjC domain-containing demethylases (KDMs), which hydroxylate lysine residue on histones, and the TET (10–11 translocation) family of 5-methylcytosine hydroxylases, which induce DNA demethylation of CpG islands near gene promoters, thus causing multifaceted epigenetic rearrangements that can further contribute to neoplastic growth [86]. Moreover, TRAP1 down-regulates the activity of cytochrome *c* oxidase, the complex IV of the respiratory chain [71]. Altogether, these observations are in accord with a model positioning TRAP1 as a primary bioenergetic regulator that matches oxygen availability with the respiratory response of cells. In the stressful conditions of a growing tumor mass, TRAP1 participates in the metabolic switch toward aerobic glycolysis, decreasing OXPHOS activity while enhancing glucose utilization [33,87], and it also tunes redox homeostasis and the metabolic interplay between mitochondria and the rest of the cell. This retrograde signalling directed outside of mitochondria is exemplified by the oncometabolic effects of succinate and by the concomitant inhibition of autophagy and enhancement of motility mastered by TRAP1 when cancer cells are in a low nutrient environment [88,89]. TRAP1 inhibition also elicits the mitochondrial unfolded protein response (UPRmt), a program characterized by the induction of

mitochondrial chaperone and protease genes in response to impaired mitochondrial proteostasis [90].

The role of metabolic rheostat exerted by TRAP1 goes beyond modulation of OXPHOS components. Lipid metabolism is frequently deregulated in cancer cells, with an increase in fatty acid synthesis to boost proliferation that is accompanied by a diminished fatty acid oxidation (FAO). Mice with genetic defects in FAO caused by ablation of long-chain 3-hydoxy acyl-CoA dehydrogenase (LCHAD) develop hepatic steatosis and HCC and have increased TRAP1 levels already at preneoplastic stages [91]. Contrasting results indicate that TRAP1 deficiency increases FAO [71], indicating that mechanistic studies are required to better understand the connection between TRAP1 activity and lipid metabolism in neoplastic models. Pilot experiments also show that TRAP1 intervenes in glutamine utilization. In a panel of cell models, genetic inhibition of TRAP1 induces the use of glutamine to increase glutathione levels, to replenish TCA intermediates and to increase the rate of oxygen consumption [44]. Altogether, these changes could allow TRAP1 knock-out cells to cope with increased oxidative stress and to compensate down-modulation of glucose metabolism.

3.4. Moulding a versatile TRAP(1)

TRAP1 activity is finely modulated by several post-translational modifications (PTMs) that constitute a way to connect it to environmental changes, providing rapid and flexible responses. S-nitrosylation at residue Cys501 promotes TRAP1 degradation [92], elicits conformational changes to distal sites in the protein structure, affects chaperone cycle dynamics and decreases its ATPase activity, possibly increasing cell sensitivity to death stimuli [93]. Oncogenic kinase pathways directly phosphorylate TRAP1. TRAP1 tyrosine phosphorylation in a c-Src-dependent way favors cytochrome oxidase inhibition [71]. Data showing that overexpression of TRAP1 increases ERK phosphorylation [94] and that activation of BRAF enhances TRAP1 serine-phosphorylation [95] linked the chaperone to the Ras/Raf/ERK signalling pathway. These observations have been placed in a mechanistic perspective when it was demonstrated that a mitochondrial fraction of ERK forms a complex with TRAP1 and SDH in models of neurofibromin-deficient cells, where hyperactivation of the Ras pathway promotes neoplastic growth. The ERK/SDH/TRAP1 multimeric complex enhances TRAP1 activity and the consequent SDH inhibition, whereas mitochondrial ERK is maintained active by the chaperone and TRAP1 inhibition abrogates cell tumorigenicity [81].

TRAP1 also interacts with the mitochondrial deacetylase SIRT3. In GSCs, TRAP1 activity sustains stemness and metabolic plasticity by a reciprocally activating interaction with SIRT3 that increases respiration and lowers ROS. In this model, TRAP1 would stabilize SIRT3 that in turn would preserve the chaperone activity of TRAP1 by deacetylating it. Several OXPHOS components in GSCs directly bind to the TRAP1/SIRT3 complex, the inhibition of which markedly diminishes the expression of some respiratory complex components [74]. Therefore, it can be argued that OXPHOS activity is controlled by chaperone and deacetylase activities. NAD^+ activates SIRT3, and a major regulator of the mitochondrial NADH/NAD^+ ratio is NADH dehydrogenase, the respiratory chain complex I. Hyperactivation of Ras/ERK signalling, as in the case of neurofibromin loss, dampens expression and activity of NADH dehydrogenase, lowering both respiration and intracellular NAD^+ levels. In neurofibromin-deficient cells, both NAD^+ and SIRT3 activity interfere with *in vivo* tumorigenicity, and this anti-neoplastic effect is synergistic with TRAP1 inhibition [96]. In this tumor cell model, both SIRT3 and TRAP1 inhibition increase the enzymatic activity of SDH to a similar extent and without any additive effect, which is strongly suggestive of a common effector mechanism. It must be underlined, though, that this is in contrast with the proposed activation of TRAP1 by SIRT3 in GSCs [74]. Notwithstanding these inconsistencies, which could be context-dependent, these observations suggest that SDH is a point of intersection between the bioenergetic effects caused by SIRT3 induction and TRAP1 ablation. We can forecast a complex scenario where Ras/ERK-mediated inhibition of NADH dehydrogenase, the unbalance of mitochondrial NADH/NAD^+ ratio, SIRT3 and SDH enzymatic activities together with the chaperone functions of TRAP1 form a network of interactions that link the bioenergetic features and the neoplastic potential of cells (Fig. 2). Observations that pharmacological inhibition of NADH dehydrogenase elicits BRAFV600E melanoma cell death by increasing ROS levels and inducing PTP opening, and that TRAP1 overexpression antagonizes this treatment [97], further add to this fascinating drawing and suggest therapeutic possibilities [98,99].

4. TRAP1 targeting

The first approach pursued to inhibit TRAP1 was based on the high degree of homology with the ATP binding pocket of the cognate chaperone Hsp90. The Hsp90 ATPase inhibitor 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) was modified by adding mitochondrial targeting moieties such as 1–4 tandem repeats of cyclic guanidinium or triphenylphosphonium (TPP) [100]. These modular molecules, called Gamitrinibs, accumulated in tumor cell mitochondria and displayed anti-neoplastic activity in prostate cancer models [101]. Even if the active group of Gamitrinibs also inhibits Hsp90, these compounds can show specificity for TRAP1, as it is much more abundant than Hsp90 in mitochondria of a number of cancer cells [102]. In a similar approach, TPP was conjugated to the Hsp90 inhibitor PU-H71. The resulting molecule, PU-H71-TPP, elicited mitochondrial depolarization and apoptosis in acute myeloid leukemia cells [103]. Crystal structures of human TRAP1 complexed to PU-H71 guided the design of another TPP-binding derivative, SMTIN-P01, which accumulates in mitochondria and is cytotoxic in cancer cells [102]. SMITN-P01 analogs conjugate PU-H71 to TPP with spacers of different lengths, showing unexpected effects. The compound with a 10-carbon linker (SMTIN-C10) established both orthosteric and allosteric interactions with TRAP1, inducing structural changes in the chaperone from open to closed state. Most interestingly, SMTIN-C10 is an activator of TRAP1 ATPase activity but perturbs TRAP1 function, decreases protein levels of the clients SDHB and SIRT3 and has anticancer activity both *in vitro* and *in vivo* [104]. In the case of Gamitrinibs, orthosteric inhibition of TRAP1 ATPase activity maintained the chaperone in an open conformation that is still capable of interacting with clients, such as CyPD, whereas the linker region of SMTIN-C10 determines a different mode of drug binding and action, leading to client binding inhibition [98,99].

It is important to highlight here that the effects exerted by the TPP group *per se* on the target cell are poorly understood, and that inhibiting the ATP-binding pocket of TRAP1 inevitably displays some degree of off-target effect on the other components of the Hsp90 chaperone family. Hence, finding highly selective TRAP1 inhibitors is an urgent issue [98,99]. In a screening to find TRAP1 inhibitors with a reduced effect on cytosolic Hsp90 without using any mitochondriotropic moiety, a molecule called DN401 in which the purine ring of the Hsp90 inhibitor BIIB021 was modified to a pyrazolopyrimidine scaffold was identified. DN401 showed TRAP1 selectivity and exhibited anticancer activity [105].

A totally different strategy is the search for allosteric TRAP1 inhibitors by rational design. Such molecules would modulate TRAP1 ATPase activity by binding an allosteric site distal from the ATP binding pocket. Molecular dynamics simulations of TRAP1 were run and the dynamic coordination between any two residues throughout the structure was assessed as a function of the fluctuation of their distance [40,106]. In this framework, highly coordinated residues are characterized by low pair-distance fluctuations. As a consequence, groups of connected residues move cooperatively and thus have the highest probability of controlling TRAP1 functional motions. This approach allowed identifying and characterizing a putative allosteric site in the large sub-region of the middle domain that is responsible for the structural reorganization following ATP hydrolysis. This information was

translated into pharmacophore models to screen drug databases, and eleven small molecules were selected. All of them displayed a selective inhibition of TRAP1 ATPase activity with no significant effects on Hsp90 and reverted TRAP1-dependent downregulation of SDH activity in cancer cells, reproducing the effects of TRAP1 genetic ablation. Three of these inhibitors abrogate *in vitro* tumorigenic growth of highly malignant peripheral nerve sheath tumor (MPNST) cells [98]. This same computational approach was extended to investigate whether the natural product honokiol, which is a potential antineoplastic and a SIRT3 activator, can affect TRAP1 activity. The honokiol derivative HDCA (honokiol bis-dichloroacetate) turned out to interact with the same allosteric pocket of TRAP1, inhibiting both its activity and the tumorigenic growth of malignant cells and paving the way for a novel class of TRAP1 inhibitors [107].

Importantly, it is possible to envisage a combined use of TRAP1 inhibitors with other anti-neoplastic effects, causing synergic effects. First evidences indicate that Gamitrinibs augment efficacy of MAP kinase inhibitors in models of BRAFV600E melanoma, an effect observed also on drug-resistant melanoma cells [108]. c-Myc inhibition with JQ1 and OTX015, two molecules that target the BET family protein of transcriptional regulators, induces apoptosis of malignant glioma cell in synergy with Gamitrinibs [109]. The combined use of Gamitrininb and histone deacetylase inhibitors (romidepsin and panobinostat) enhances apoptosis of glioblastoma cells also in patient-derived xenografts [110].

5. Conclusions

Understanding the biochemical functions of TRAP1 and how they reverberate on the metabolism, survival and growth of tumor cells is a rapidly evolving field of study. Next steps to disentangle the complexity of this issue will require the integration between different approaches of investigation. These encompass fine dissection of the molecular dynamics features of the chaperone, elucidation of the mode of client engagement by TRAP1 under different biological conditions and dissection of the downstream impacts of these interactions on bioenergetics and (epi)genetic tuning in cancer cells. Combining this knowledge with the development of highly selective and effective TRAP1 inhibitors will open exciting perspective of tailoring therapeutic interventions in specific cancer settings.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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