The role of TRAP1, the mitochondrial Hsp90 in cancer progression and as a possible therapeutic target

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Hsp90, a 90 kDa heat shock protein (HSP), is a molecular chaperone involved in various cellular processes. It is highly conserved across species and plays a critical role in protein folding quality control, protein degradation and, most importantly, stabilizing proteins against heat stress. Emerging evidences suggest that HSPs accumulate not only in stressful conditions, but also in pathophysiological conditions and tumours. They play a role in refolding partially damaged functional proteins and also stabilize cell survival factors. Studies also suggest the role of organelle-specific Hsp90 chaperones in these processes, which further adds to the complexity. These findings make the Hsp90 family a potent target for anticancer drugs. Tumour necrosis factor receptor-associated protein 1 (TRAP1), the mitochondrial homolog of Hsp90, is found to play a pivotal role in mitochondrial bioenergetics, maintenance of mitochondrial integrity and mounting stress responses. Tumour cells exhibit a peculiar phenotype known as the Warburg effect, where they evade the mitochondrial oxidative phosphorylation and produce ATP by aerobic glycolysis. Studies suggest TRAP1 as a key regulator in this metabolic switchover along with a pivotal role in drug resistance and antiapoptotic effects. This article discusses the molecular mechanisms of TRAP1 to regulate cancer growth, its role in protecting cells from apoptosis and toxicity from anti-cancer drugs. The possibility of TRAP1 as a potential target for cancer therapies in the near future based on new-age therapeutic strategies by inhibiting the protein is also discussed here.

Keywords: Apoptosis, cancer progression, drug resistance, heat shock proteins, tumour cells.

THE response of whole organisms or cultured cells when exposed to heat stress is universal. They all show an elevated synthesis of a family of stress-induced proteins[1]. Heat shock proteins (HSPs) were first discovered by the Italian scientist Ferruccio Ritossa, while demonstrating the results of temperature-induced puffing pattern of salivary gland chromosomes of *Drosophila busckii*[2]. He observed that the puffing patterns were not only due to a temperature jump, but also how quickly a given temperature threshold was achieved. A similar puffing pattern was seen when the salivary glands were incubated with dinitrophenol and sodium salicylate. The 'puffing' pattern was reasoned to be due to gene activation following heat stress, thereby enhancing expression of specific proteins. Therefore, these proteins were named heat shock proteins[3].

HSPs are a large family of molecular chaperones involved in a wide array of functions, conferring thermotolerance to cells and quality control in the endoplasmic reticulum (ER), which leads to the activation of unfolded protein response (UPR). HSPs play an indispensable role in assisting protein folding and refolding of misfolded or damaged proteins when cells are subjected to stress conditions[4,5]. Thus, HSPs have a protective role in cells, but their overexpression has a profound effect leading to many pathological conditions. Since HSPs promote cell survival and have antiapoptotic effects, their role in cancer is undeniable[6].

HSPs are categorized according to their molecular mass. As the number of members in various HSP families expanded, there were discrepancies which led to confusion until Kampinga *et al.*[7] devised a novel way of classification. The 90 kDa HSP family (Hsp90 or HSPC), found in eubacteria and all branches of eukaryotes, but not in archaea, constitutes 1–2% of cellular proteins under non-stress conditions[8,9]. The cytosolic isoforms of Hsp90 include Hsp90α and Hsp90β (ref. 8). Another isoform, Hsp90N, involved in the neoplastic transformation was also identified[10]. The 94 kDa glucose-regulated protein, Grp94, is an analogue of the cytosolic Hsp90, primarily residing in the ER[8,11].

Another molecular chaperone that bears a strong homology to Hsp90 was first identified using the yeast two-hybrid system while screening for proteins that bind to the N-terminal region of the type-1 receptor for tumour necrosis factor (TNFR-1IC)[12]. This chaperone protein, known as the TNF receptor-associated protein 1 or Hsp75, has a mitochondrial localization sequence in the amino terminal and is localized in the mitochondria, specifically in the mitochondrial matrix[13,14]. The TRAP1 transcript of ~2.4 kb was originally found to be expressed in normal tissues but more prominently in tumour cell lines[12]. Amino acid sequence analysis revealed a significant shared homology between TRAP1 and Hsp90 family members with 34% sequence identity, including conserved substitutions with about 60% overall homology[12]. The highest homology was found in the amino terminal[13].

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There are also structural and functional differences between TRAP1 and other Hsp90 members. TRAP1 lacks the highly charged domain present in Hsp90 (ref. 12) and it does not bind to Hsp90 cp-chaperones, p23 and Hop (p60) (ref. 13).

TRAP1 is involved in a wide range of cellular processes[15–17]: (a) modulating mitochondrial dynamics and inducing metabolic switch over; (b) regulating UPR in the ER, during ER stress and promoting cell survival; (c) controlling the cell cycle to modulate cell proliferation and maintaining stemness, and (d) protecting cells from drug-accumulated cytotoxicity and evading cell death through its antiapoptotic activities.

Considering the intricate role of the Hsp90 family chaperones in the regulation of a wide range of cellular processes involved in tumour growth and survival by mediating adaptivity to stress conditions and activation of other oncogenic proteins, HSPs are one of the prime targets in cancer therapy[18]. The majority of Hsp90 inhibitors bind to the N-terminal domain to prevent ATP binding and hydrolysis, thereby obstructing client protein processing. The clients are then subsequently targeted for proteasomal degradation. The bacterial-derived benzoquinone ansamycin geldanamycin (GM), capable of binding to the ATP-binding site of Hsp90, emerged as the first inhibitor of Hsp90 proteins[19]. In this context, the ATP-binding pocket of TRAP1 is also located in the N-terminal. This ATP-binding domain also possesses ATPase activity[12,13], thus strengthening the similarities between TRAP1 and Hsp90. C-terminal-binding inhibitors like coumarin-containing DNA gyrase inhibitor, novobiocin (Nvb) and its analogues 4- or 7-tosylcyclonovobiocic acid (4TCNA or 7TCNA) prepared by removing noviose moiety and introducing a tosyl subunit at C-4 or C-7 coumarins, showed promising results in antiproliferative assays and also downregulation of critical Hsp90-dependent signalling proteins like HER2 and Raf-1 in various breast cancer cell lines[20,21]. Novobiocin inhibitors also produced antitumorigenic effects in melanoma cells. A novel novobiocin-derived Hsp90 C-terminal binding inhibitor, KU135, reduced Hsp levels and induced G_2/M cell cycle arrest and apoptosis in melanoma cells[22]. There are also TRAP1-specific inhibitors like shepherdin and gamitrinib[18] the details of which and the challenges encountered during their development will be discussed in this article.

The roles played by TRAP1 in the growth of tumours are still covered under a shroud of enigma, primary among them being whether TRAP1 acts as an oncogene or oncosuppressor. Here our scope is to delve further into the available literature and check whether TRAP1 can serve as a pharmacological target for cancer therapy development in the near future.

TRAP1: structure

The TRAP1 gene is located on chromosome 16p13.3, which encodes the TRAP1 mRNA of 2223 nucleotides from a single locus, according to current annotations in the NCBI database. This TRAP1 mRNA translates into a precursor protein of 704 amino acids, including the mitochondrial import sequence of 59 amino acids, which is removed upon entering the mitochondrial matrix[23].

The basic structure of Hsp90 is conserved across homologs. It is found in a homodimeric form, with each protomer consisting of three major domains: the N-terminal domain (NTD), which binds to ATP and mediates ATP hydrolysis, the C-terminal domain (CTD), which provides the dimerization interface between the protomers, and the middle domain (MD) that aids in ATP hydrolysis[24–26]. It also contains a conserved arginine 380 residue that does not necessarily participate in ATP hydrolysis, but acts as an ATP sensor. This stabilizes the NTD–MD conformation to mediate efficient ATP hydrolysis[27]. However, the charged linker and the C-terminal MEEVD motif of eukaryotic cytosolic Hsp90 are lacking in TRAP1. Alternatively, TRAP1 has a mitochondrial localization sequence. The NTD of TRAP1 shares the Bergerat fold, which is commonly found in Hsp90 chaperones. Several conserved motifs characterize the Bergerat fold. Among them, Motif I (Glu 115) acts as the catalytic glutamate and activates a water molecule to attack the γ-phosphate of ATP[24].

Crystal structure of TRAP1 revealed an ordered 14-residue extension of the N-terminal β-strand. Although absent in yeast and bacteria, this extension or 'strap' is present in most eukaryotic Hsp90 proteins, including the cytosolic and organellar homologs and is known to cross between protomers in the closed state. The strap displays a regulatory function, controlling the conformation changes from apo (open) and ATP-bound (closed) states, and is thereby responsible for the ATPase rates being temperature-dependent[26]. This N-terminal strap acts as a thermally sensitive kinetic barrier with the TRAP1 homodimer actively reaching a closed state and increased ATP hydrolysis of nearly 200-fold between 25°C and 55°C (ref. 28).

A 2.3 Å resolution crystal structure of TRAP1 in its catalytically active closed state revealed an asymmetric conformation of the TRAP1 homodimer. This asymmetry allows the two-step ATP hydrolysis mechanism, which drives client remodelling and client release. The N-terminal strap regulates ATP hydrolysis by stabilizing the closed state through trans-protomer interactions[25].

TRAP1: roles in neoplastic growth

Tumour cell metabolism

The glucose metabolism differs in tumorigenic and non-tumorigenic cells. In non-tumorigenic cells, glucose metabolism under aerobic conditions occurs via aerobic glycolysis followed by mitochondrial oxidative phosphorylation (OXPHOS), which produces the bulk of ATP. The result is the net production of 30 or 32 ATP molecules from one molecule of

glucose[29]. The end-product of aerobic glycolysis, pyruvate, enters the TCA cycle and finally the coenzymes NADH and $FADH_2$ enter the electron transport chain where the free energy from electron transport drives ATP synthesis. Tumorigenic cells bypass the mitochondrial OXPHOS, rather pyruvate from glycolysis is converted to lactate. The net result is two ATP molecules produced from one molecule of glucose[30–33].

In 1924, Otto Warburg while studying tumour cell metabolism in rats, reported a rather peculiar aspect which became the primary focus of many researchers in the field of cancer biology in the years to come. The data he collected showed that tumour cells have a high uptake of glucose and produce large amounts of lactic acid, compared to non-tumorigenic cells[34]. Warburg reported that tumour cells have a respiratory impairment in which oxidation and phosphorylation are not coupled, resulting in ATP production through fermentation even in the presence of oxygen[35]. Increased lactic acid levels result in increased generation of H^+ ions and pumping them to the environment, thus decreasing the pH. Acidification of the tumour microenvironment facilitates extracellular matrix (ECM) breakdown and promotes metastasis[36].

Recent evidences have challenged the Warburg effect as a general trait of all tumours. Some tumour cells, specially the slow cycling ones, depend on mitochondrial OXPHOS rather than glycolysis[37] and the tissue of origin and tumour microenvironment determine the metabolic pathways for cancer cells[38]. Recent studies have brought the TRAP1 regulation of tumour metabolic pathways to new light[16,17,39–41].

The first evidence of metabolism regulation by TRAP1 was reported by Sciacovelli *et al.*[42]. TRAP1 binds to both complex IV (cytochrome oxidase, COX) and complex II (succinate dehydrogenase, SDH), and it inhibits the enzymatic activity of SDH or succinate-coenzyme Q reductase (SQR). TRAP1 knockdown (KD) SAOS 2 osteosarcoma cells (shTRAP1 cells) transfected with mouse TRAP1 cDNA (shTRAP1 + mTRAP1 cells) showed inhibited SQR activity. Mouse embryonic fibroblast (MEF) cells stably expressing TRAP1 showed lowered-SQR activity and treatment with 17-allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of TRAP1 activity, reactivated SQR activity in MEF cells. In stage-IV human colorectal cancer samples with lymph node metastases and stage I–III samples, TRAP1 was upregulated with concomitant reduced SQR activity compared to normal mucosa.

Oxygen consumption rate (OCR) was lowered in SAOS-2 osteosarcoma cells expressing elevated TRAP1 levels compared to shTRAP1 cells. Addition of uncoupler FCCP (carbonyl cyanide-p-trifluoromethoxy phenylhydrazone) elevates respiration well above the basal level in shTRAP1 cells, indicating an increased respiratory capacity. On the contrary, the control cells having already utilized their maximal respiratory capacity under basal conditions are insensitive to FCCP treatment. OXPHOS is the primary source of ATP in shTRAP1 cells, whereas the primary source of ATP in control cells is glycolysis[42].

$TRAP1^{-/-}$ (knockout, KO) murine adult fibroblasts (MAFs) had a higher basal OCR and a notably high maximum respiratory rate accompanied by decreased glycolysis when compared to WT mice. In accordance with this, glycolytic metabolites were reduced and TCA cycle metabolites (e.g. α-ketoglutarate and citrate), anaplerotic substrate propionylcarnitine, fatty acid oxidation and NAD^+/NADH ratio were increased in TRAP1 KO cell lines. Steady-state ATP levels were high in TRAP1 KO cell lines and induced expression of TRAP1 significantly lowered ATP levels in TRAP1 KO cell lines. Activity of mitochondrial complex IV was also increased in TRAP KO MAF cell lines compared to WT mice[43]. Human neuroblastoma (IMR-32) TRAP1 KD, TRAP1 overexpressing (OE) and parental cell lines were studied. OCR levels of these cell lines were in the order: TRAP1 OE < parental < TRAP1 KD. OXPHOS inhibition using rotenone did not have any effect on TRAP1 OE cells[44].

Cancer cells also tend to utilize glutamine as a carbon source that fuels the TCA cycle. Tumour cells vary in their glutamine requirements ranging from glutamine auxotrophs relying on glutamine-mediated TCA cycle anaplerosis to those that do not require exogenous glutamine to proliferate[45]. Total glutamine and glutamate levels showed a significant increase in TRAP1 KO HEK2937 and A549 cells, indicating an anaplerotic shift in metabolism[46]. Another study demonstrated a stark contrast in intracellular glutamine and glutamate levels among TRAP1 KO and OE IMR-32 cells, with the OE cells showing a four-fold increase, signifying TRAP1 involvement in metabolic rewiring[44]. Glutamine deprivation in TRAP1 KD background reduces cell motility significantly[47] and TRAP1 interacts with both glutaminase and glutamine synthetase. Inhibition of TRAP1 by gamitrinib-triphenylphosphonium (G-TPP) in glutamine-dependent non-small cell lung cancer (NSCLC) cells increases glutamine synthetase activity resulting in the arrest of cell growth[48], while mitoquinone treatment reduces expression of glutaminase[49].

Increased succinate levels along with detectable levels of HIF1α were seen in TRAP1 expressing SAOS-2 osteosarcoma cells[42]. Hypoxia activates the hypoxia-inducible factor 1 (HIF1) transcription factor complex[50]. During normoxia, the HIF1α subunit of HIF1 is ubiquitously degraded upon prolyl hydroxylation, but during hypoxia prolyl hydroxylation is inhibited and the HIF1α subunit and HIF1 transcriptional activity are stabilized[30]. HIF1 activates genes for glucose import (*GLUT1*, *GLUT3*) and enzymes involved in glucose breakdown (phosphofructokinase1, aldolase1), thus upregulating glycolysis. HIF1 also targets pyruvate dehydrogenase kinase 1 (PDK1) which inactivates pyruvate dehydrogenase (PDH), thus blocking pyruvate from entering the TCA cycle[51]. Succinate accumulation inhibits HIF1α prolyl hydroxylases, thereby stabilizing HIF1α (ref. 52). Thus, TRAP1 initiates a feedback loop by inhibiting SDH

and respiration (OXPHOS) that induces HIF1, which further blocks ATP production by OXPHOS[42].

TRAP1 was found to interact with mitochondrial c-src and inhibit its kinase activity in the process. Mitochondrial c-src and TRAP1 have opposing effects on mitochondrial OXPHOS with TRAP1 expressing cells regulating c-src activity resulting in the low OCR phenotype[43].

Phosphofructokinase (PFK) catalyses a key irreversible step in glycolysis, converting fructose-6-phosphate to fructose-1,6-bisphosphate. PFK is inhibited by high ATP and citrate levels[53,54]. Since TRAP1 deficiency results in a high energy state, levels of fructose-1,6-bisphosphate were low in TRAP1 KO cell lines[43]. TRAP1 also directly upregulates PFK-1 levels, thus enhancing the glycolytic phenotype[55]. Inhibiting the mitochondrial ATP synthase with oligomycin shifted the ATP production pathway to glycolysis in both TRAP1 KO and WT cell lines[43]. Thus, reinforcing the hypothesis that deregulated mitochondrial OXPHOS causes ATP production to shift towards aerobic glycolysis.

TRAP1 modulates mitochondrial dynamics: fission and fusion

Mitochondrial morphologies are not static when viewed in live cells. Mitochondria change their shapes and distribution frequently, termed as mitochondrial dynamics. They change their shape primarily by fission and fusion. Mitochondrial fission and fusion are mediated by membrane remodelling mechanochemical enzymes, belonging to the dynamin family of large GTPases. Fission is mediated by dynamin-related/like protein (Drp1/Dlp1), recruited by Mid49, Mid51 and Mff in mammals. Mitofusin (Mfn) and optic atrophy 1 (Opa1) mediate fusion of the outer and inner membranes respectively[56,57]. Increased fusion is associated with higher OXPHOS during proliferation, whereas fission disconnects TCA and OXPHOS[57].

Mitochondrial metabolism is deregulated in tumour cells and TRAP1 plays a key role as discussed earlier. In this context, altered metabolism in tumour cells has been correlated with dysfunctional mitochondrial dynamics[58,59]. TRAP1 modulates mitochondrial metabolism and hence the question arises whether TRAP1 also has a role in the modulation of mitochondrial dynamics.

Immunofluorescent microscopy showed TRAP1 KD SH-Sy5y neuroblastoma cells with tubular-shaped mitochondria and fragmented mitochondria in the control cells. Drp1 and Mff expression levels were decreased in TRAP1 KD cells and overexpression of TRAP1 in KD cells reversed this effect. There was no change in the expression levels of fusion proteins; Mfn1/2 and Opa1 were unaltered in the TRAP1 KD cells[60]. Human neuroblastoma IMR-32 cells stably transfected phenotypes TRAP1 OE and TRAP1 KD, along with parental cells were compared to study TRAP1 expression effects in mitochondrial dynamics. TRAP1 OE cells displayed increased mitochondrial fission and decreased mitochondrial fusion, whereas the reverse was seen in TRAP1 KD cells. The results were consistent with IMARIS analysis, where smaller and increased mitochondria numbers were seen in TRAP1 OE cells. TRAP1 localization in the mitochondria was also found to be essential for mitochondrial fission.

The mitochondria of invasive and metastatic MDA-MBL231 breast cancer cells expressing low levels of TRAP1 were rod-shaped and overexpression of TRAP1 in these cells changed the mitochondrial phenotype to tubular network, suggesting an induction of mitochondrial fusion[61,62].

TRAP1 protects cells from oxidative stress, regulates UPR, induces drug resistance and inhibits apoptosis

A cell is said to be under oxidative stress when there is an imbalance of reactive oxygen species (ROS) generation and limited antioxidant defences. The primary intracellular sources of ROS are the mitochondria[63] and mitochondrial HSP90, TRAP1 plays a role in reducing oxidative stress.

Yoshida *et al.*[43] reported elevated ROS in TRAP1 KO murine adult fibroblasts (MAFs) compared to WT MAFs. Sciacovelli *et al.*[42] reported that TRAP1 reduces the enzymatic activity of complex II (SDH) of mitochondrial OXPHOS. TRAP1 KD SAOS-2 osteosarcoma cells and human cervix carcinoma HeLa cells showed increased levels of intracellular ROS and mitochondrial superoxide anion. This was also noticed when the cells were cultured in a nutrient-starved medium (containing pyruvate and glutamine, but no serum and glucose), followed by induction of cell death. It was concluded that TRAP1 interaction with SDH-A catalytic site decreases ROS levels, since the SDH unit is important for ROS generation[64]. Deferoxamine (DFO), an iron chelator, is a potent inhibitor of mitochondrial function and homeostasis. Treatment of normal human hepatocyte cell line with DFO showed inhibitory effects on TRAP1. The TRAP1 mRNA and protein levels were decreased with a concomitant increase in ROS levels[65]. Increased TRAP1 expression was associated with elevated levels of scavenging tripeptide GSH in diethylmaleate (DEM) adapted SAOS-2 osteosarcoma cells, resistant to increasing ROS levels[66].

The link between TRAP1 and apoptosis was demonstrated by Hua *et al.*[67]. TRAP1 silencing results in enhanced ROS production mediated by granzyme M and accelerated release of cytochrome *c*. Cytochrome *c* release is an essential signal for mitochondria-mediated apoptosis and together with apoptotic protease-activating factor (Apaf1) and procaspase 9, it activates caspase 3 initiating the apoptotic cascade[40,68]. Granzyme M decreases mitochondrial membrane potential ($\Delta\psi m$), disrupting the mitochondrial permeability transition (mPT) and also increasing ROS levels[67]. Granzyme M cleaves ICAD activating CAD, and triggering caspase-dependent apoptosis leading to DNA damage with a typical laddering pattern. It also degrades PARP

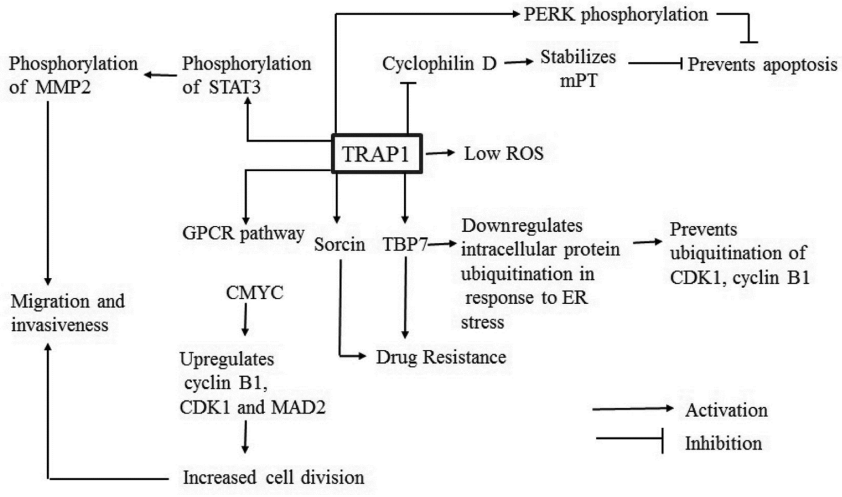

Figure 1. TRAP1 roles in cancer progression are not only confined to cellular metabolism. TRAP1 interacts with SDH-A catalytic subunit of complex IV of OXPHOS to downregulate ROS levels, and prevents cellular death by inhibiting cyclophilin D while promoting phosphorylation of PERK. TRAP1 interaction with sorcin and TBP7 confers drug resistance and also protects proteins from proteasomal degradation. GPCR activity and STAT3 pathway are also induced by TRAP1 resulting in increased expression of cell cycle mediators and activation of MMPs respectively, leading to cell migration and invasiveness.

preventing cellular DNA repair and inducing apoptosis[69]. Granzyme M-induced death was reversed and ROS levels decreased following TRAP1 OE (ref. 67).

TRAP1 controls apoptosis by a variety of processes, the primary among them being regulating mPT. Also, mPT is an important step in cell death as it induces mitochondria swelling, outer membrane rupture and initiates apoptosis by cytochrome *c* release[70]. TRAP1 antagonizes immunophilin cyclophilin D (CypD) activity, directing a protective effect on the cells by stabilizing mPT and inhibiting cell death by its ATPase activity[71]. CypD seems to control mPT by regulating mitochondrial permeability transition pore (mPTP) opening, as seen in CypD OE cells with increased swelling and spontaneous cell death[70]. Under conditions of oxidative stress, p53 translocates to the mitochondrial matrix, where it transiently binds to TRAP1, freeing CypD from its inhibiting effects. CypD now acts on structural mPTP proteins inducing mPTP opening[72]. Mitochondrial Ca^{2+} uptake also has a role in mPTP opening. Ca^{2+} uptake in the mitochondria increases ROS levels and leads to ROS-dependent mPT onset. Anoxia/reoxygenation induces increased Ca^{2+} uptake in the mitochondria, which leads to increased ROS levels, disrupting mPT and finally cell death[73]. Ca^{2+} binds to the F1 domain of F1F0ATPase synthase in a CypD-dependent manner triggering mPTP opening[74]. Prolonged mPTP opening has harmful consequences leading to elevated oxidative stress and loss of mitochondrial homeostasis[75]. TRAP1 inhibition sensitizes cells to CypD-mediated mPT and eventually causes cell death[71,72]. TRAP1 expression was seen to strengthen in response to hypoxia and contribute to increased hypoxic cell viability and preserved $\Delta\psi m$ (ref. 76). TRAP1 regulation of mPTP opening was also illustrated in cardiomyocytes and diabetic rats with high glucose uptake[76,77].

Upon treatment of human leukaemia Hl60 cells and human lung cancer DMS114 cells with β-hydroxyisovaleryl-shikonin (β-HIVS), a compound known to induce apoptosis, cell death was seen along with decreased TRAP1 levels and increased release of cytochrome *c*. β-HIVS inhibits TRAP1 and causes mPTP opening leading to the apoptotic cascade[78]. Esophageal squamous cell carcinoma (ESCC) was also sensitive to TRAP1-induced protective effect with TRAP1 KD inducing increased ROS levels, mitochondria depolarization, cell-cycle arrest at G_2/M and apoptosis[79]. Apoptosis inhibition was also seen in NSCLC cell lines with high TRAP1 expression that correlated with poor prognosis[80]. T-cell acute lymphoblastic leukaemia (T-ALL) with inactive polycomb repressive complex 2 (PRC2) was reported[81] to be apoptosis-resistant. This apoptotic-resistant phenotype is also mediated by TRAP1, as PRC2 loss results in the upregulation of CRIP2, which subsequently activates TRAP1. TRAP1 expression also correlates with decreased activation of caspases, leading to the antiapoptotic phenotype[66] (Figure 1).

The role of ER in mediating apoptosis is increasingly being validated. Unfolded proteins enter the ER for proper folding and subsequent transport to their respective destinations. The homeostasis of protein folding is suitably maintained by the ER, and when this intracellular homeostasis is challenged as seen during ER stress, the ER initiates UPR. The UPR sets in motion three responses: (a) reduction of protein load entering the ER, (b) increasing capacity of the ER to handle increased protein load and (c) when the ER stress exceeds a certain threshold, the above

two responses fail to mitigate, thus triggering transcriptional upregulation of Bcl_2 homology 3 (BH3) only apoptotic proteins leading to apoptosis[82,83]. TRAP1 is involved in UPR and protects the cells from ER stress-induced cell death, as has been demonstrated by different studies. Takemoto *et al.*[84] observed that upon ER stress, levels of ER-resident caspase 4 were elevated in TRAP1 KD cells. ER stress uses both the survival and apoptotic pathways, mediated by ER-resident chaperone GRP78/Bip and c/EBP homologous protein (CHOP) respectively, and TRAP1 regulates both of them. TRAP1 KD upregulates GRP78/Bip and downregulates CHOP, and cells with TRAP1 KD are subjected to increased apoptosis. This is seen after 24 h of early phase ER stress, which indicates that the role played by TRAP1 in GRP78/Bip and CHOP regulation possibly delays ER stress-induced cell death. Amoroso *et al.*[85] discovered a novel role of TRAP1 along with proteasome regulatory protein TBP7, both of which colocalize and interact in the ER, and regulate intracellular protein ubiquitination in response to ER stress which was further reiterated by TRAP1/TBP7 interference experiments which increases ubiquitinated protein levels and sensitizes cells to ER stress-induced apoptosis. Matassa *et al.*[86] further studied the role of TRAP1 in UPR and found that it was involved in a translational role in colon carcinoma cells. TRAP1 interacts with members of the translational apparatus and regulates protein synthesis through the eIF2α pathway. The selective stress-responsive proteins are upregulated, protecting against dangers due to ER stress.

TRAP1 was upregulated in human colorectal carcinoma cells (hCRC) and was associated with drug resistance to 5-fluorouracil (FU), oxaliplatin (1-OHP) and irinotecan (IRI)[87]. Sorcin, a Ca^{2+}-binding protein, interacts with TRAP1 via its NH_2 terminal and TRAP1 sorcin-binding enhances the multidrug resistance observed in hCRC cells[88] and breast cancer cells[89]. Resistance to the chemotherapy drug cisplatin (DDP) and inhibition of apoptosis was seen in human lung carcinoma cell lines A549 and H1299. TRAP1 was elevated in A549/DDP and H1299/DDP cells rather than A549, and H1299 cells and knockdown of TRAP1 resulted in apoptosis activation and increased ROS levels and decreased $\Delta\psi$m (ref. 90).

Bortezomib and anthracyclin-resistant cell lines displayed high levels of TRAP1 along with inhibited apoptosis. Importantly, the TRAP1/TBP7 pathway seemed pivotal in maintaining the drug-resistant phenotype, as confirmed by TBP7 mutant cell lines with re-established drug sensitivity. TRAP1 KD cells showed low levels of PERK phosphorylation which is involved in the activation of genes controlling cell metabolism and cytoprotective functions[91]. Paclitaxel resistance along with inhibited apoptosis was observed in thyroid carcinoma cells[92] and breast cancer cells[89] expressing high TRAP1 levels. High TRAP1 expression in ovarian cancer was inversely correlated with tumour grade, and silencing TRAP1 led to cisplatin resistance[93].

All these observations reveal that TRAP1 expression leads to tumour progression by helping cells evade apoptosis, through a variety of intracellular mechanisms and also protecting tumour cells from drug-induced toxicity leading to drug resistance, although the exact mechanism is still unclear.

TRAP1 regulation of cell cycle and tumour metastasis

The expression profile of genes involved in cell growth and metastatic ability was studied in relation to TRAP1 expression in human breast cancer cell line MDA-MB-231 and lung adenocarcinoma cell line A549. TRAP1-positive cells were associated with high levels of gene coding for cell proliferation, growth, G-protein coupled receptor pathway, angiogenesis and cell adhesion, while metastatic genes were high in TRAP1-negative cells[94]. TRAP1 binding to members of the cell cycle was first accessed by Chen *et al.*[95], who observed a 75 kDa HSP (TRAP1) with substantial sequence homology to members of the Hsp90 family bind to the simion virus 40 T-antigen binding domain of hypophosphorylated Rb with its L× C× E motif. Interestingly, Rb and TRAP1 formed complexes during the M-phase and heat shock. TRAP1 was also seen to refold denatured Rb *in vitro*[95]. TRAP1 maintains Rb1 in the hypophosphorylated form, thus inducing more Rb1 binding to E2F1. MCF7 cells with TRAP1 KD showed lowered levels of Rb1 protein and reduction in Rb1/E2F1 co-immuno-complexes with a significant increase in S-phase cell fraction[96].

Oligonucleotide microarray analysis in primary human fibroblasts after c-Myc activation revealed TRAP1 as a Myc target[97]. Proto-oncogene Myc lies at the crosstalk of several pathways regarding cellular growth[98]. Chromatin immunoprecipitation (ChIP) assay also revealed Myc accumulation in a time-dependent manner at the TRAP1 promoter in Burkitt's lymphoma and neuroblastoma cells. Myc silencing downregulates TRAP1 levels and the same results were also observed after N-Myc silencing. Myc silencing inhibited tumour cell motility and invasion which was restored following TRAP1 re-expression[99]. TRAP1 silencing resulted in the downregulation of G_2/M checkpoint regulators CDK1, cyclin B1 and spindle assembly checkpoint regulator MAD2 in colorectal, lung and breast carcinoma cell lines along with cell cycle arrest at the G_2 phase. Translocation of CDK1/cyclin B1 complex is a critical event during G_2/M transition and TRAP1-silenced cells revealed poor localization of CDK1 and cyclin B1 as conferred by immunoblot analysis and confocal microscopy. Consequently, MAD2 levels and fragmented nuclei were absent in TRAP1-silenced MCF7 cells. TRAP1 regulation of G_2/M and spindle assembly regulators is post-transcriptional mediated by TRAP1/TBP7 interaction. TRAP1/TBP7-silenced cells showed increased ubiquitination of CDK1, cyclin B1 and MAD2 (ref. 100).

A study[101] of human colorectal adenocarcinoma tissues found a correlation between TRAP1 expression and pathologic T-stage. It proposed tumour invasion of stromal tissue via EMT as a result of TRAP1 expression[101]. TRAP1 was significantly elevated in colorectal carcinoma (CRC) with lymph node metastasis (LMN) and tumour node metastasis (TNM). Furthermore, TRAP1-positive CRC patients were associated with poor prognosis[102] and low survival rate[103]. High TRAP1 levels correlated with (a) uniform intense staining pattern in lymph node metastasis prostate cancer[104]; (b) muscularis serosa infiltration, lymph node metastasis and tumour node metastasis stage III/IV with a concomitant increase in cyclin B, cyclin D1, cyclin E, MMP-2, VEGF levels in gastric cancer tissue[105]; (c) LMN and histological grade II–III and clinical stage III–IV, resulting in poor prognosis and reduced mean survival time in kidney cancer patients[106]; (d) poor tumour differentiation, advanced pT-stages, lymph node and distant metastasis, advanced FIGO stages and poor prognosis in epithelial ovarian cancer[107] and (e) client protein (TBP7, IF1α, EFIG, etc.) upregulation and poor prognosis in human metastatic CRC[108]. Ovarian cancer displayed a distinct characteristic, with low TRAP levels associated with high tumour grade and stage[93,109]. TRAP1 expression was elevated in poorly differentiated and follicular variants of papillary thyroid carcinomas. Silencing TRAP1 resulted in G_2/M cell cycle arrest in TC cell lines with concomitant downregulation of ERK signalling and low BRAF protein levels[92]. G_2/M cell-cycle arrest and decreased cell proliferation as a result of TRAP1 KD were seen in esophageal cancer cells[79], along with attenuation of tube formation in human glioblastoma multiforme cell lines[110]. Results from scratch-wound heal assay and cell-migration assay showed TRAP1 OE human neuroblastoma IMR-32 cells with enhanced cell migration compared to TRAP1 KD cells. Metastatic tumour growth was seen in nude mice subcutaneously injected with TRAP1 OE cells, while TRAP1 KD tumour xenografts showed localized tumour growth[61]. TRAP1 expression and associated pathologies were studied in the $Pten^{+/-}$ background, a common molecular abnormality that drives prostate cancer in humans. Invasive prostatic adenocarcinomas were prevalent in $Pten^{+/-}$ TRAP1 transgenic mice, while TRAP1 loss in $Pten^{+/-}$ mice lowered invasiveness. Also, $Pten^{+/-}$ TRAP1 transgenic mice displayed increased cell viability and reduced apoptosis with anti-apoptotic Bcl2 upregulation, as seen from reverse phase protein array (RPPA)[111].

TRAP1 expression correlates with lymph-node metastasis in human esophageal squamous cell cancer (ESCC). The ESCC cells expressing high TRAP1 showed drug resistance to cisplatin and inhibition of apoptosis along with increased cell motility. TRAP1 positively mediated phosphorylation of STAT3 and its target gene matrix metalloproteinase 2 (MMP2), and migration and invasiveness of the ESCC cells depended on STAT3/MMP2 pathway regulation by TRAP1 (ref. 112).

Matrix metalloproteinases (MMPs) which belong to the family of zinc-dependent extracellular matrix (ECM) remodelling endopeptidases are involved in a wide array of functions in cancer pathology. They facilitate tumour invasion and metastasis by breaking the ECM and basement membrane. MMPs cleave cell-adhesion molecules, remove sites of adhesion and expose new building sites, thus inducing epithelial-mesenchymal transition (EMT)[113]. MMPs also regulate inflammation in the tumour microenvironment and help in immune evasion, thus promoting tumour growth[114]. Expression levels of E-cadherin, vascular endothelial growth factor (VEGF) and MMPs were studied in gastric cancer (GC) tissues. E-cadherin and VEGF showed an inverse relationship with poorly differentiated GC tissues expressing high VEGF and very low E-cadherin compared to well-differentiated GC tissues, which displayed low VEGF and high e-cadherin levels. MMPs (MMP1 and MMP2) were also high in poorly differentiated GC tissues[115]. High VEGF expression was seen in GC patients with brain metastasis[116]. Cellular signal (AKT/p70S6K, BRAF/ERK and AMPK) pathways relating to cell growth and proliferation were also under TRAP1 control.

AKT/p70S6K signalling pathway: AKT is an important player in the tumorigenic phenotype[117]. Ribosomal protein S6 kinase (p70S6K), a downstream target of AKT, is involved in actin cytoskeleton organization and cell migration. p70S6k, AKT, PDK1 and p85 phosphoinositide 3-kinase (PI-3 kinase) localization along with activated mTOR enrichment were found at the actin arc, suggesting the importance of the AKT/p70S6K pathway activation in cell migration[118]. TRAP1 silencing in human HCT116 colon carcinoma cells and HEK293 embryonic kidney cells increased protein synthesis with p70S6K and RSK1 being hyper expressed and showing high phosphorylation levels. TRAP1-interfered cells showed a high migratory ability *in vitro*, possibly due to high p70S6K levels. In a cohort of 34 human colon malignancies, high TRAP1 expression was associated with low p70S6K levels, and vice versa[119].

TRAP1 control of AKT and p70S6K is post-transcriptional with TRAP1 KD HCT116 and HEK293 cells expressing high levels of phosphorylated Akt, the upstream regulator of p70S6K, which also explains the high levels of phosphorylated p70S6k observed. This is associated with enhanced cell migration under basal conditions. P70S6k induces expression of transcription factor Snail with consequent E-cadherin downregulation, while HEK293 cells show no change in Snail mRNA levels. HCT116 cells show slight upregulation of Snail with concomitant E-cadherin decrease. Notably, in nutrient-deprived conditions, cell motility had reduced significantly in TRAP1 KD cells[47].

TRAP1 expression in ovarian cancer inversely correlates with grade and stage. Silencing TRAP1 increases p70S6K phosphorylation levels, which leads to increased cell migratory capacity. TRAP1 contributes to EMT by directly

correlating to E-cadherin protein levels and inversely correlating to Slug/Snai 2 (transcription factors controlling E-cadherin expression), MMP2 and MMP9 mRNA levels[109].

Glioblastoma tissues with invasive properties were characterized by high STIP1 (phosphorylation-induced protein 1) levels, which also correlated with high TRAP1 levels. Downregulation of STIP1 resulted in suppressing the TRAP1/AKT pathway, lowering MMP2 levels and inhibiting cell invasion[120].

BRAF/ERK signalling pathway: B-type RAF proto-oncogene (BRAF), which regulates the MAPK/ERK pathway controlling cell cycle and differentiation, is one of the 12 most frequently mutated genes in the cancer background[40]. TRAP1 regulation of BRAF protein expression is at the translational levels, with TRAP1 silencing associated with decreased protein expression. This association is due to TRAP1 control of BRAF ubiquitination levels, as seen in TRAP1-silenced colorectal carcinoma cells with increased BRAF ubiquitination levels. Downregulation of BRAF upon silencing leads to attenuation of the ERK pathway, with low levels of phosphorylated ERK1/2 observed. This correlates with increased cell count in G_0/G_1 and G_2/M phases[121].

BRAF-upregulated colorectal carcinoma cells were not sensitive to oxaliplatin (1-OHP) and irinotecan (IRI)-induced apoptosis; this anti-apoptotic phenotype was dependent on TRAP1 (ref. 122). $BRAF^{V600E}$ gene mutation in papillary thyroid carcinoma (PTC) correlated with TRAP1 expression, both of which jointly led to the development of PTC[123]. TRAP1 regulation of the ERK pathway was also seen in mouse NIH/3Y3 cell line. Overexpression of TRAP1 resulted in increased levels of ERK phosphorylation leading to rapid proliferation[124]. TRAP1 and ERK1/2 are also involved in mitochondrial bioenergetics regulation. ERK1/2 is localized in the mitochondria and phosphorylates TRAP1, which further maintains ERK1/2 activity in the mitochondria, inhibiting complex II of mitochondrial OXPHOS and prolyl hydrolases, decreasing ATP production by OXPHOS, thus contributing to the Warburg phenotype[125].

AMPK signalling pathway: AMPK (adenosine 5′-monophosphate (AMP)-activated protein kinase) is a nutrient-sensing molecule involved in regulating bioenergy metabolism[17,40]. Glucose-starved human glioblastoma LN229 cells-induced activation of the energy sensor AMPK along with autophagy induction and TRAP1 expression dampened the AMPK activation, as demonstrated by TRAP1 silencing experiments resulting in exacerbation of the energy-deprived responses along with reduced levels of ATP production. TRAP1 also inhibited AMPK substrate UNC-51-like kinase (ULK1) and preserved cytoskeletal dynamics, mediating the release of cell motility effector FAK (focal adhesion kinase), thus promoting cell motility under low nutrient conditions[126]. Treatment with gamitrinib or knocking down TRAP1 resulted in AMPK phosphorylation and mTORC1 inhibition[127].

Role of TRAP1 in the maintenance of stemness

Cancer stem cell (CSC) population poses a big road block in cancer therapy. These self-renewing tumorigenic fractions are the major drivers of relapses and metastasis dissemination. Molecular chaperones (namely Hsp90, Grp78, TRAP1) play a part in the formation and maintenance of CSC phenotype[128]. The role of TRAP1 in the maintenance of CSC via regulating the Wnt/β-catenin pathway was first reported by Lettini *et al.*[129]. TRAP1 expression significantly correlates with stem cell markers (CD166, CD133, CD44) in colorectal carcinoma CSCs. The shTRAP1 HCT116 cells display a low profile of CD166 expression along with significant impairment in colony-formation. TRAP1 mRNA levels and several Wnt/β-catenin-related genes are co-expressed with TRAP1 silencing, resulting in the attenuation of the Wnt-β-catenin pathway and loss of stem-like phenotype in HCT116 cells. TRAP1 modulation of the Wnt/β-catenin pathway is mediated by the regulation of β-catenin ubiquitination/phosphorylation by the TRAP1/TBP7 network. High-grade glioblastoma tumour exhibits high Sox2 expression (a marker of stemness), correlating with high TRAP1 expression. TRAP1 is responsible for the regulation of cell metabolism and reduced ROS production in glioma stem cells (GSCs), and maintains stemness and self-renewal properties of GSCs. TRAP1 interacts with mitochondrial deacetylase sirtuin-3 (SIRT3) and both are involved in the inhibition of ROS production and metabolic adaptation of GSCs to survive in nutrient-deprived conditions[130]. High TRAP1 expression in neural stem cells preserves $\Delta\psi$m, protects them from microglia-induced neurotoxicity, inhibits CypD-dependent mPTP opening and cytochrome *c* release, thus inhibiting apoptosis[131].

TRAP1 in cancer therapy: development of TRAP1-specific inhibitors and their efficacy

TRAP1 protects cells from oxidative stress, regulates UPR, induces drug resistance and inhibits apoptosis (Figure 2). TRAP1 localization in the mitochondria modulates mitochondrial dynamics[61], regulates tumour cell metabolism and maintenance of Warburg phenomenon[42,43], inhibits apoptosis, enhances cell proliferation and protects cancer cells from drug-induced toxicity[67,87,100]. All these attributes make TRAP1 an attractive therapeutic druggable target for cancer therapy.

One of the first criteria in the development of TRAP1-specific inhibitors is intracellular mitochondrial accumulation, and peptidomimetic shepherdin emerged as the first agent that could accumulate inside the tumour mitochondria in the intermembrane space, the inner membrane and matrix. Shepherdin localization in the mitochondria is due to the presence of antennapedia helix-III homeodomain cell-penetrating sequence. Shepherdin induces loss of $\Delta\psi$m and release of cytochrome *c*, triggering cell death in Raji

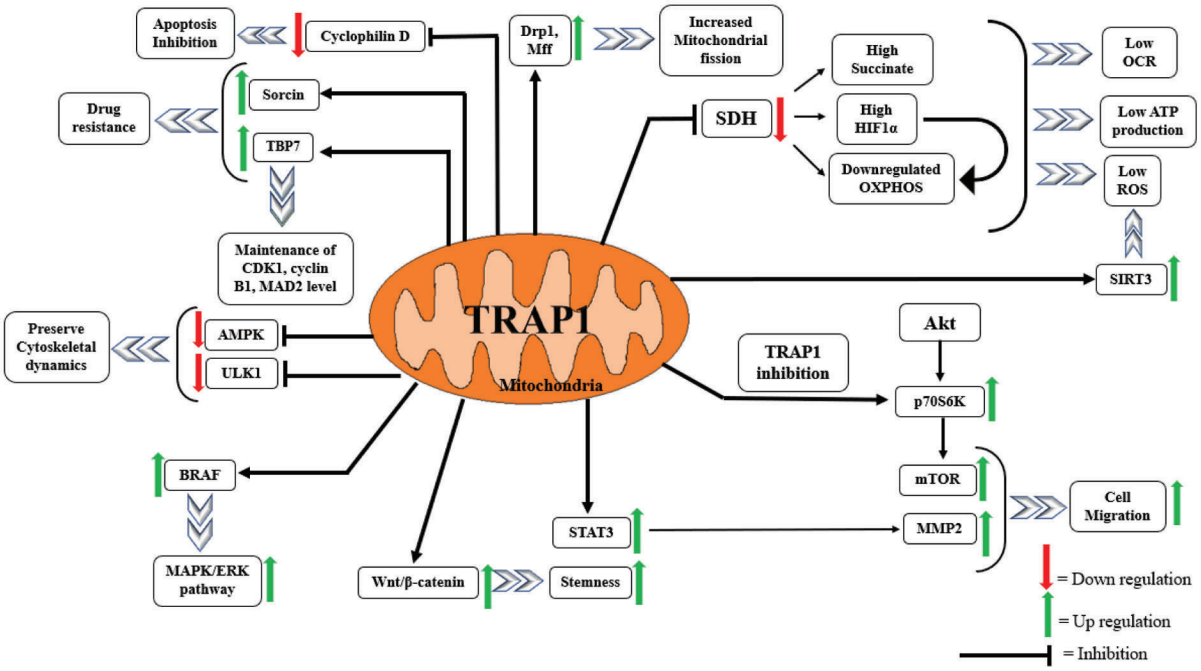

Figure 2. TRAP1 interactions contributing to the tumour phenotype. TRAP1 promotes aerobic glycolysis by downregulating complex II of mitochondrial OXPHOS, succinate dehydrogenase, which leads to succinate and HIF1α accumulation thereby decreasing ATP production and ROS levels. TRAP1 blocks cyclophilin D activity and protects tumour cells from apoptotic death. It also promotes drug resistance by interacting with sorcin and TBP7. It increases cell-cycle activity and contributes to stem cell character by preventing ubiquitination of various proteins, including cyclin B1, BRAF and β-catenin to name a few. TRAP1 inhibition increases p70S6K levels resulting in increased cell migration, which is also increased via its interaction with STAT3.

lymphoblastoid cells. The effects of shepherdin were prominent in transformed cells exhibiting high levels of mitochondrial Hsp90 (TRAP1), as seen in normal NIH3T3 fibroblasts versus Ras-transformed NIH3T3 fibroblasts[71]. Shepherdin was originally designed to interact and inhibit the surviving–Hsp90 interaction. Survivin, a 16.5 kDa member of the mammalian inhibitor of apoptosis (IAP) protein family, is involved in apoptosis inhibition from both the intrinsic and extrinsic pathways, with mitochondrial cell death being the most well-documented. Strong experimental evidences also suggest survivin association in cell division and overexpression of survivin has been observed in most tumours[132]. Survivin also interacts with the N-terminal of Hsp90 and Hsp90-binding helps in stabilizing survivin[133]. Shepherdin binds to the N-terminal region of Hsp90, also known as the ATPase activity site and disrupts the surviving–Hsp90 complex. Cervical carcinoma Hela cells treated with shepherdin result in cataclysmic loss of cell viability and cytochrome *c* release inducing mitochondrial cell death, in a concentration-dependent manner. Shepherdin binding to the ATP pocket of Hsp90 destabilizes Hsp90 client proteins, including survivin, Akt, Bcl2, CDK-4 and CDK-6 while unaffecting the levels of Hsp90, Hsp70 or PCNA. Shepherdin also exhibits a selective antitumour activity. HeLa cells or prostate carcinoma cells display loss of cell viability while normal human fibroblasts display no such cell viability loss under similar concentrations of shepherdin. These data are also consistent with *in vivo* studies[134]. Shepherdin induction of mitochondrial death is mediated by mPTP opening in a CypD-dependent manner, due to its interaction with the mitochondrial pool of Hsp90, TRAP1 (ref. 135). Glioblastoma cells treated with shepherdin inhibit TRAP1 action and result in the sudden loss of $\Delta\psi$m, cytochrome *c* discharge through mPTP opening in a CypD-dependent manner and also suppress glioma growth *in vivo*. Concentration-dependent degradation of Hsp90 client proteins, including Akt, IAP proteins, survivin and Bcl2 has been observed, while no significant changes in Hsp70 levels were seen even for higher shepherdin concentration. Shepherdin accumulates both in the cytosol and mitochondria, and inhibits both Hsp90 and its subcellular fractions[136]. Drug-resistant human colorectal carcinoma cells, when treated with shepherdin, again become sensitive to FU, 1-OHP and IRI. Shepherdin antagonizes TRAP1-mediated drug resistance and induces apoptosis[87]. Hsp70/Hsp90 and survivin destabilization, and MMP-2 activity suppression were seen in shepherdin-treated retinoblastoma cells along with apoptosis induction[137].

Kang *et al.*[138] designed a new class of Hsp90 inhibitors, gamitrinibs (geldanamycin mitochondrial matrix inhibitor), with selective mitochondrial localization. Gamitrinib contains a benzoquinone ansamycin backbone derived from Hsp90 inhibitor 17-(allylamino)-17-demethoxy-geldanamycin (17-AAG), a linker region at the C17 position, and 1–4 tandem repeats of cyclic guanidinium (gamitrinib-G1-G4) or triphenylphosphonium (gamitrinib-TPP-OH) which

acts as the mitochondrial targeting moiety. Gamitrinib accumulation in the mitochondria of transformed cells was high and the 17-AAG portion was found to bind readily to the mitochondrial Hsp90 ATPase pocket to induce sudden loss of the inner mitochondria membrane potential along with the rapid discharge of cytochrome *c*, dependent on CypD. These effects were minimal in normal cells, thus highlighting the selective action of gamitrinib. Apoptosis was induced with the death of the entire cell population observed after 24 h. However, gamitrinib-induced apoptosis was independent of the pro-apoptotic Bcl2 protein Bax. Gamitrinib also did not affect the levels of Hsp90 client proteins in the cytosol nor upregulate Hsp70 levels[138], as seen with shepherdin treatment[134,136]. Hsp70 promoted tumorigenesis by inducing drug resistance, reducing mPT and inhibiting apoptosis[139]. Glioblastoma cells treated with sub-cytotoxic levels of gamitrinib exhibited upregulation of multiple chaperones involved in mitochondrial UPR, induction of stress response transcription factor, CHOP and its dimerization partner C/EBPβ within 6 h of treatment. However, treatment with mitochondriotoxic concentration of gamitrinib that directly leads to collapse of mitochondrial integrity and cell death did not show any upregulation of mitochondrial UPR protein networks[140]. Gamitrinib also inhibited localized tumour growth, prevented metastasis in prostate adenocarcinoma[141] and reduced cell viability with increased apoptosis, inhibition of foci and colony formation in BRAF-mutated and drug-resistant colorectal carcinoma cells[122].

The major hindrance in the development of TRAP1 selective inhibitors is due to highly conserved active site residues and almost superimposable nucleotide-binding site structures observed in the Hsp90 homologues. Purine scaffold Hsp90 inhibitor (PU-H71) was conjugated with the mitochondria targeting moiety triphenylphosphonium (TPP). The N9 alkane of Pu-H71 purine ring was substituted with alkylated TPP to design an active and TRAP1-specific inhibitor, SMTIN-P01, with a 100-fold mitochondrial accumulation. SMTIN-P01 exhibited the general traits of TRAP1 inhibitors showing inhibition of TRAP1 ATPase activity higher than gamitrinib and inducing mitochondria membrane depolarization. SMTIN-P01 like gamitrinib did not modulate Hsp90 client proteins Chk1 and Akt, while Hsp70 was also not upregulated[142]. Another purine scaffold Hsp90 inhibitor, BIIB021 was modified, and the purine ring of BIIB021 was changed to pyrazolopyrimidine scaffolds to produce a highly specific TRAP1 inhibitor, DN401. Dn401 inhibition of cytosolic Hsp90 was low, which was the primary roadblock in earlier inhibitors. Low mitochondrial accumulation of Hsp90 inhibitors was due to strong binding to cytosolic Hsp90 and DN401 produced the opposite results. Signature TRAP1 inhibition responses were observed – loss of $\Delta\psi$m, increase in ROS levels, cytochrome c release and apoptosis. Hsp90 client proteins were not effectively degraded and Hsp70 was not induced; cytotoxicity in normal cells was also low compared to Hsp90 inhibitors. *In vivo* prostate cancer xenografts treated with DN401 displayed reduced tumour growth[143]. Aminopyrimidine NVP-HSP990 is an oral inhibitor with specificity to both Hsp90 and TRAP1. NVP-HSP990 binds to the N-terminal ATP binding domain of Hsp90 and shows more than 90% inhibition of TRAP1 ATPase activity with an IC_{50} value of 320 nmol/l. GTL-16 human gastric adenocarcinoma tumour cell line was selected for NVP-HSP990 administration due to its dependency on C-Met, which is also a client protein of Hsp90. C-met levels were decreased and Hsp70 was induced in a dose-dependent manner following NVP-HSP990 treatment, while Akt and ERK levels were also decreased along with attenuation of cell proliferation. NVP-HSP990 activity was studied in gastric carcinoma, breast cancer, acute myelogenous leukaemia and human non-small cell lung carcinoma xenograft models, and anti-tumour effects were observed in each of them[144]. NVP-HSP990 downregulated TRAP1 interactor sorcin and members of the translational machinery, increased ubiquitination of BRAF and lowered Erk phosphorylation. It also inhibited eIF2Fα phosphorylation, a master regulator of protein synthesis in human colorectal carcinoma cells[121]. BRAF and ERK levels were lowered and cell cycle arrest was observed in NVP-HSP990-treated thyroid carcinoma cells[92].

Certain plant-derived compounds also exhibited anti-TRAP1 activity. B-HIVS, a compound isolated from the medicinal herb *Lithospermum radix* inhibited TRAP1 expression and induced apoptosis[78]. Green tea polyphenols (GTE) treated human pancreatic ductal adenocarcinoma HPAF-II cells showed apoptosis induction, growth suppression and inhibition of Akt. The results were due to GTE-mediated inhibition of Hsp chaperones, notably Hsp90, Hsp75 (TRAP1) and Hsp27 (ref. 145). The vegetal derivative honokiol and its lipophilic bis-dichloroacetate ester, honokiol DCA (HDCA), were found to bind selectively to TRAP1 and inhibit its ATPase activity. HDCA reversed TRAP1 downregulation of SDH and increased SQR activity in a SIRT3-dependent manner. It also increased mitochondrial superoxide levels and prevented tumour growth[146].

Combination therapy is widely used in cancer treatment, which boosts the anticancer activity of tumour drugs. Gamitrinib along with TRAIL (TNF-α-related apoptosis inducing ligand), a death receptor agonist, efficiently killed cancer cells while not affecting normal ones. TRAIL plus gamitrinib combination accelerated loss of inner mitochondrial membrane potential, release of cytochrome *c* and induced activation of caspase 3 and 7 very early after treatment[140]. Gamitrinib and genotoxic drug doxorubicin combination therapy showed synergistic enhancement of cytotoxicity in cervix, ovary, glioblastoma, prostate and lung carcinoma cell lines. There was an enhanced accumulation of pro-apoptotic Bim and Bax proteins in the mitochondria and prostate cancer xenograft models treated with this combination showed inhibited tumour growth[147]. TRAP1 inhibitor gamitrinib and histone deacetylases (HDAC1/HDAC2) inhibitor romidepsin or panobinostat combination treatment enhanced

Table 1. TRAP1 expression in various tumour types

Tumour type	TRAP1 expression	Reference
Lung	High levels of expression correlated with shorter recurrence-free survival	80
Prostate	High in metastatic population and low in benign population	104
Thyroid	High in poorly differentiated thyroid carcinoma	92
Esophagus	High in poorly differentiated carcinoma with increased lymph node metastasis	79, 112
Colorectal, gastric and kidney	High expression correlated with lymph node metastasis, with advanced pathologic T stage	87, 101–103, 105, 106, 108
Glioblastoma	High	110
Breast	High in non-invasive MCF-7 cells and low in metastatic MDA-MB-231 cells	62, 89
Ovarian	TRAP1 expression inversely correlated with high tumour grade and stage	93, 109
Cervical and bladder	Expression correlates inversely with tumour stage	43

cell death through modulation of pro- and anti-apoptotic proteins in the glioblastoma cells while reducing tumour growth for *in vivo* treatments[148]. Gamitrinib and liver X receptor (LXR) agonist LXR623 combination treatments inhibited tumour growth for *in vitro* and *in vivo* treatments in a broad range of solid tumours[149].

Novel strategies involving nanocarriers are implicated in the design of TRAP1 inhibitors. Iron-oxide nano particles (IONs) were conjugated with geldanamycin (GA)-FITC with a mitochondria localization sequence (MLS) tagged. The resulting compound ION–GA–FITC with an attached MLS peptide was stable and was observed to selectively bind TRAP1 in the mitochondria, modulating the TRAP1 response to mitochondria metabolism and dynamics involved in tumour growth[150]. D'Annessa *et al.*[151] reportedly designed allosteric compounds that stimulate Hsp90/TRAP1 ATPase activity displaying anticancer properties in low micromolar to nanomolar range. Structure and conformational dynamics-based allosteric ligands could also be considered as a novel therapeutic strategy for selective TRAP1 targeting[152].

There are challenges in the development of TRAP1-specific inhibitors mainly due to the high homology with Hsp90 family members. A good understanding of the structural dynamics of TRAP1 is necessary and will pave the way for the development of highly specific compounds that could bind and inhibit the action of TRAP1 while preserving other Hsp90 chaperones and client proteins.

Conclusion

Tumour cells regulate their metabolism by cycling between high glycolysis state and low OXPHOS state or low glycolysis state and high OXPHOS state, and TRAP1 plays a key role in this switchover. TRAP1 is upregulated in many cancer types (viz. lung, breast, thyroid, prostate, esophagus, colorectal, glioblastoma) (Table 1)[62,79,80,87,89,92,101–104,108,110,112], while some cancer types (ovarian, cervical, renal)[43,93,109] show low TRAP1 expression. Recently, Yu *et al.*[153] adopted a systems biology approach by analysing data from the Cancer Genome Atlas, single-cell transcriptomics data as well as extensive study of the available literature to develop a model linking OXPHOS and glycolysis. They postulated that cancer cells have three stable states based on the activity of HIF1 and pAMPK: (i) high HIF1and low pAMPK leading to the Warburg state; (ii) low HIF1and high pAMPK leading to the OXPHOS state and (iii) high HIF1 and high pAMPK leading to a hybrid state[153].

The hybrid state indicates the condition where both glycolysis and OXPHOS are active. This phenotype significantly helps cancer cells to adapt to changing conditions in the tumour microenvironment (TME), such as hypoxia or nutrient starvation, and increases their overall survival and progression[153]. The hybrid state also correlates with the fact that some metastatic cell lines (ovarian and breast) have low levels of TRAP1, resulting in high OXPHOS[62,93,109]. Also, cancer cells utilize glutamine as a carbon source, which helps fuel the TCA cycle anaplerosis[45].

TME regulates the metabolic phenotype of the tumour cells. There are multiple factors regulating the switchover with TRAP1 at the helm, but there are still many aspects of TRAP1 regulation that remain unanswered.

While mitochondrial metabolism is one aspect of TRAP1 involvement in cancer progression, modulation of mitochondrial dynamics is an area that needs further studies. TRAP1 is also involved in inhibiting apoptosis and drug resistance by interacting with a host of downstream targets and protecting proteins from proteasomal degradation. Protection from cell death is mediated primarily by lowering the levels of ROS generation, which also confers tumour cells with a high migratory potential in nutrient-deprived conditions. While a low TRAP1 environment results in high phosphorylation of p70S6K and enhanced cell migration, the same was not observed after glutamine removal. In contrast, high TRAP1 expressing cells displayed increased cellular mobility after glutamine removal. This leads to the conclusion that TRAP1 could be essential in protecting cells under stress conditions and also confer them with a high migratory ability under such conditions.

The role of TRAP1 in maintaining the hybrid state, particularly its interaction with AMPK, which recently is being studied for its contribution to promoting tumorigenesis[154], is still unclear. Also, catabolism of glutamine, which fuels the TCA cycle in low nutrient conditions, and an increase in glutamine and glutamate levels was seen in the TRAP1 KO background[46], which suggests that there may be some

other factors along with TRAP1, which help cancer cell replenish the ATP requirement and promote their progression.

TRAP1 regulation of cell cycle and tumour metastasis is complicated, with a vast range of cellular crosstalk with TRAP1 at the helm mediating the progression of the tumour phenotype. The present study analyses the varied and complex role of TRAP1 in regulating the progression of cancer while posing some unanswered questions that need further exploration. While the advancement of TRAP1-specific drugs is opening new avenues in cancer therapy, the use of nano-carriers as an effective tool for the targeted delivery of drugs can also prove useful in future therapeutic approaches.

Conflict of interest: The authors declare that there is no conflict of interest.

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