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MINI REVIEW

Past, present, and emerging roles of mitochondrial heat shock protein TRAP1 in the metabolism and regulation of cancer stem cells

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Abstract Tumor necrosis factor receptor-associated protein 1 (TRAP1), a member of the HSP90 family, controls a variety of physiological functions, including cell proliferation, differentiation, and survival. Most studies have been devoted to understanding the anti-apoptotic roles of TRAP1 in cancer and targeting it for tumor control in clinical settings. Additionally, we have identified a new role for TRAP1 in regulation of liver regeneration after partial hepatectomy in TRAP1 transgenic mice and cellular proliferation in TRAP1-overexpressing cells, via mitochondrial alterations. Moreover, recent works have indicated a role for TRAP1 in the regulation of cancer stem cells (CSCs) as well as a metabolic switch between mitochondrial respiration and aerobic glycolysis called as "Warburg effect." This review discusses the implications of TRAP1 action for both metabolism and the regulation of CSCs.

Keywords TRAP1 · Apoptosis · Fatty liver · Cancer stem cells · Warburg effect

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Introduction

Cells use a variety of mechanisms to defend themselves against extracellular and intracellular stresses and to maintain homeostasis. When proteins are newly synthesized or misfolded, molecular chaperones act to prevent their aggregation and maintain protein homeostasis. The most well-studied molecular chaperones are the heat shock protein, 70 kDa (HSP70s) (Li et al. 2000; Shim et al. 2002) and their co-chaperones, such as DnaJ/HSP40 (Laufen et al. 1999) and BCL-2-associated athanogene 3 (BAG3)/BCL2-interacting cell death suppressor (BIS), which regulate stress-induced cell death (Lee et al. 1999; Yoo et al. 2014). Another molecular chaperone, HSP90, is a global cellular regulator critical for the folding and regulation of a variety of proteins within cells. There are different homologs of Hsp90 in mammalian cells: cytosolic HSP90, tumor necrosis factor receptor-associated protein 1 (TRAP1, also known as mitochondrial chaperone HSP75 (Felts et al. 2000)), and endoplasmic glucose-regulated protein, 94 kDa (GRP94) (Johnson 2012). Like HSP70, HSP90 also functions with several co-chaperones, such as FK506 binding protein (FKBP) and Hsp70-Hsp90 organizing protein (HOP). In addition, cooperation has been well documented between HSP70 and HSP90 and their co-chaperones in the regulation of client proteins (Riggs et al. 2004).

In 1995, TRAP1 was first identified by Song et al. as an Hsp90 family member, in a yeast two-hybrid screen for proteins associated with the type 1 tumor necrosis factor receptor-1 (TNFR1) (Song et al. 1995). That study revealed that TRAP1 mRNA was expressed variably in skeletal muscle, liver, heart, brain, kidney, pancreas, lung, and placenta, as well as in a transformed cell line, suggesting that TRAP1 is involved in a variety of cellular functions. Since then, TRAP1 has been investigated extensively, in roles associated with apoptosis, cancer, and neuronal diseases such as Parkinson's

disease (PD). In addition, we recently suggested a new role for TRAP1 in regulating both liver regeneration after partial hepatectomy (Im et al. 2013) and cellular proliferation via mitochondrial alteration (Im et al. 2007; Im and Seo 2014). TRAP1 interacts with several proteins, including retinoblastoma protein (Chen et al. 1996), cyclophilin D (CypD) (Kang et al. 2009), the calcium-binding protein Sorcin (Landriscina et al. 2010b), and the proteasome regulatory particle TBP7 (Amoroso et al. 2012). In this review, we summarize the roles of TRAP1 identified to date and provide insight into emerging roles for TRAP1 in metabolism and in the regulation of cancer stem cells.

Roles of TRAP1

Previously, TRAP1 was found to be involved in diverse cellular processes, particularly those associated with apoptosis. Several functions of TRAP1 are summarized in Fig. 1. As for other HSPs, the involvement of TRAP1 in the proliferation and apoptosis of cancer cells has also been widely investigated. However, recently presented evidence has illustrated other aspects of TRAP1 function involved in regulation of neuronal disease, metabolism, and stemness.

Proliferation and senescence

After careful investigation, HSP75 (an alternate name for TRAP1) was shown to interact with retinoblastoma protein (RB) during M phase and after heat shock, suggesting that it chaperones RB and regulates the cell cycle (Chen et al. 1996). Moreover, expression analysis with microarrays showed that MYC regulates genes involved in growth and cell cycle including TRAP1 during differentiation (Coller et al. 2000). Liu et al. analyzed differentially expressed genes and found high levels of cell proliferation-promoting genes coding for G protein-coupled receptors, cell adhesion genes, and genes associated with Rho-kinase pathways (Liu et al. 2010). TRAP1 is overexpressed in specimens of esophageal squamous cell carcinoma (ESCC), particularly in poorly differentiated tumors (Tian et al. 2014), as well as in human colorectal carcinomas (CRC) (Condelli et al. 2015). Moreover, TRAP1 knockdown induced increased reactive oxygen species (ROS) and mitochondrial depolarization, together with arrest in G2/M phase (Tian et al. 2014). The above findings indicate that targeting TRAP1 has the potential to regulate proliferation of cancer cells via regulating ROS generation as well as cell cycle-related proteins such as RB and MYC.

In contrast to cellular proliferation, senescence is a state of irreversible cell cycle arrest in which cells display characteristic and drastic morphological and metabolic changes. It is reasonable to assume that when chaperones or co-chaperones no longer function, many proteins remain misfolded, leading to a variety of dysfunctional processes within cells. For example, silencing BIS, a known co-chaperone of HSP70, induced a senescence-like phenotype in glioblastoma cells (Lee et al. 2014). We previously reported that iron chelation using deferoxamine led to decreased TRAP1 in human Chang liver cells, together with increased ROS generation, caveolin-1 (CAV1) expression, and senescence-associated beta-galactosidase (SA beta-gal) activity, the representative phenotypes of cellular senescence (Fig. 1). Overexpression of TRAP1 ameliorated the SA beta-gal activity, CAV1 expression, and ROS-related proteins such as manganese superoxide dismutase induced by deferoxamine as well as ROS generation (Im et al. 2007), indicating that TRAP1 might be associated with the upstream pathway of ROS regulation. In addition, we found that ROS levels were increased in TRAP1-overexpressing mouse NIH3T3 fibroblasts, together with activation of extracellular signal-regulated kinase (ERK) and proliferation as shown in Fig. 2 (Im and Seo 2014). These cells also display distinct morphology as well as the dramatic decrease of PGC1-α levels, indicating that TRAP1 is linked to mitochondrial biogenesis. TRAP1 plays a key role in protecting mitochondria against damaging stimuli by reducing ROS generation. For instance, granzyme M, an orphan granzyme, can cause mitochondrial swelling and mitochondrial depolarization and directly cleaves TRAP1, abolishing its antagonistic function against ROS and resulting in intracellular ROS accumulation (Hua et al. 2007). ROS seem to be a double-edged sword; they can accelerate cellular proliferation, but higher levels of ROS lead to apoptosis. This may give rise to this discrepancy between our finding and observations from Tian group, although there are also differences in cellular context and methodology. A recent review also highlighted the role of mitochondrial homeostatic mechanisms, mitochondrial metabolites, and ROS generation in signaling pathways leading to the induction and maintenance of cellular senescence and contributing to the aging process (Correia-Melo and Passos 2015). Therefore, TRAP1 may influence these intracellular processes via regulation of ROS generation.

Apoptosis

Apoptosis is the process of programmed cell death that is important for homeostasis of cells and tissues in multicellular organisms. During apoptosis, biochemical events lead to morphological and molecular alterations and finally to cell death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Mitochondria play a critical role in this process, in which Bcl-2 family members such as Bcl-2 and BAX interact reciprocally (Li and Dewson 2015). As a chaperone in mitochondria, it is not surprising that TRAP1 might play a role in apoptosis. Moreover, TRAP1 is overexpressed in cancers and implicated in drug resistance (Costantino et al. 2009;

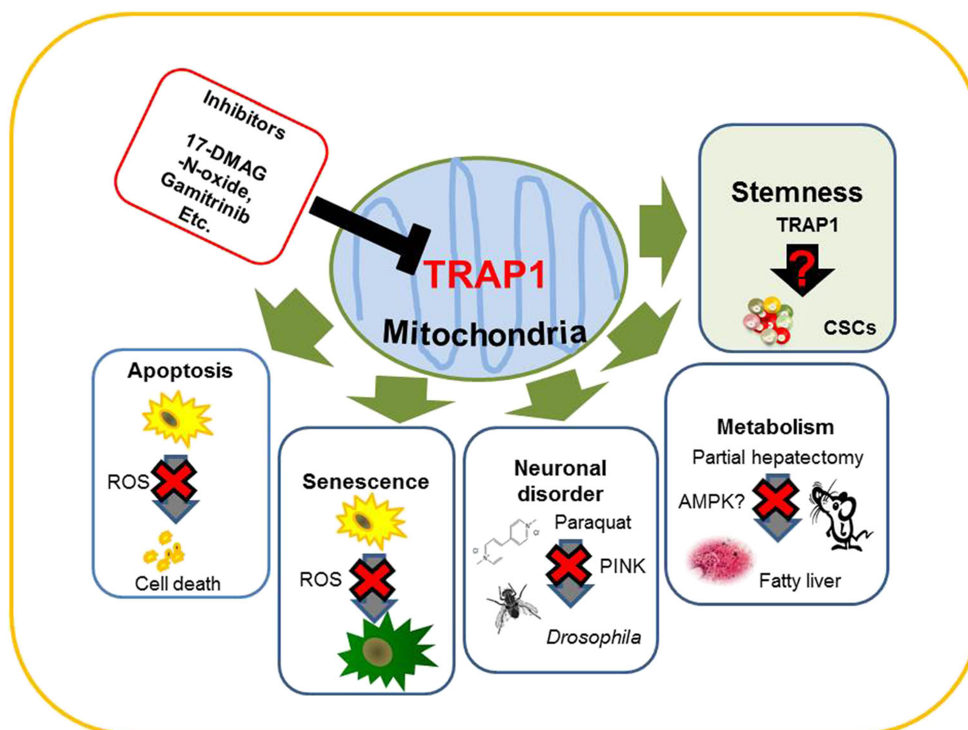

Fig. 1 Emerging roles of TRAP1 in metabolism and stemness. TRAP1 prevents apoptosis induced by various stressors. TRAP1 downregulation by iron chelation leads to a cellular senescence-like phenotype. In Drosophila, TRAP1 increases survival of paraquat-induced toxicity. In TRAP1-transgenic mice, partial hepatectomy leads to fatty liver. The role of TRAP1 in stemness is not yet clear. Current mitochondria-targeting HSP90 inhibitors suppress both HSP90 and TRAP1, requiring the development of specific TRAP1 inhibitors

Maddalena et al. 2013). In 2007, Hua et al. first showed a link between TRAP1 and apoptosis (Hua et al. 2007). They demonstrated that it antagonizes ROS generation and protects cells from granzyme M-mediated apoptosis (Fig. 1). This is consistent with our own work in which we found that overexpression of TRAP1 ameliorated iron chelation-induced senescence via ROS in Chang cells (Im et al. 2007) or cardiomyocytes (Zhang et al. 2015b). Interestingly, Takemoto et al. suggested a relationship between TRAP1 in mitochondria and the unfolded protein response (UPR) in the endoplasmic reticulum (ER) (Takemoto et al. 2011). They found that TRAP1 knockdown activated the ER-resident caspase-4, which is activated by ER stress; it also increased the basal level of GRP78 expression, which protects cells, and decreased the basal level of C/EBP homologous protein (CHOP), which induces cell death, even under ER stress. This study indicated that the mitochondria could be a potential regulator of the UPR in the ER via mitochondrial TRAP1. Subsequently, increasing evidence indicates that TRAP1 is a critical regulator of apoptosis (Masuda et al. 2004; Tian et al. 2014). Amoroso et al. demonstrated that TRAP1 and the proteasome regulatory particle TBP7 interact in the ER and control cellular ubiquitination of specific mitochondrial proteins. TRAP1 and/or TBP7 interference enhanced stress-induced cell death and increased intracellular protein ubiquitination. These data confirmed that TRAP1 functions not only in the mitochondria but also in the ER to help refold damaged proteins and prevent apoptosis (Amoroso et al. 2012). Another study in human breast cancer cells showed that TRAP1 is found in the ER, where it plays a role in protecting tumor cells against DNA-damaging agents such as anthracyclin by modulating the protein kinase RNA-like ER kinase (PERK) pathway (Sisinni et al. 2014).

Moreover, inhibiting both TRAP1 and related HSPs may be an effective strategy to manipulate cancers, although this has only been demonstrated at the cellular level (Zhang et al. 2011). Zhang's group administered green tea extract (GTE) to human pancreatic ductal adenocarcinoma HPAF-II cells and performed two-dimensional gel electrophoresis of the cell lysates. HSP90 and TRAP1, as well as HSP27, were identified among 32 proteins with significantly altered expression. These findings suggested that GTE inhibited multiple molecular targets and provided further evidence of green tea activity against pancreatic cancer. Another recent report demonstrated that TRAP1 targeted by the mitochondria-directed HSP90 chaperone inhibitor gamitrinib induced apoptosis and inhibited colony formation in BRAF-driven CRC cells (Condelli et al. 2015). Similarly, TRAP1 has recently been shown to be aberrantly upregulated in breast tumors relative to control tissues. TRAP1 knockdown downregulates mitochondrial aerobic respiration, sensitizes cells to lethal stimuli, and inhibits tumor growth in representative breast cancer cells in vivo, suggesting that TRAP1 is required for tumorigenesis. Moreover, this study showed that TRAP1 regulates mitochondrial morphology, demonstrating that lower TRAP1 levels were associated with rod-shaped mitochondrial phenotypes in invasive and metastatic MDA-MB-231 breast cancer cells. Conversely, higher TRAP1 levels were associated with a tubular network mitochondrial phenotype in non-invasive MCF-7 cells, linking TRAP1-regulated mitochondrial

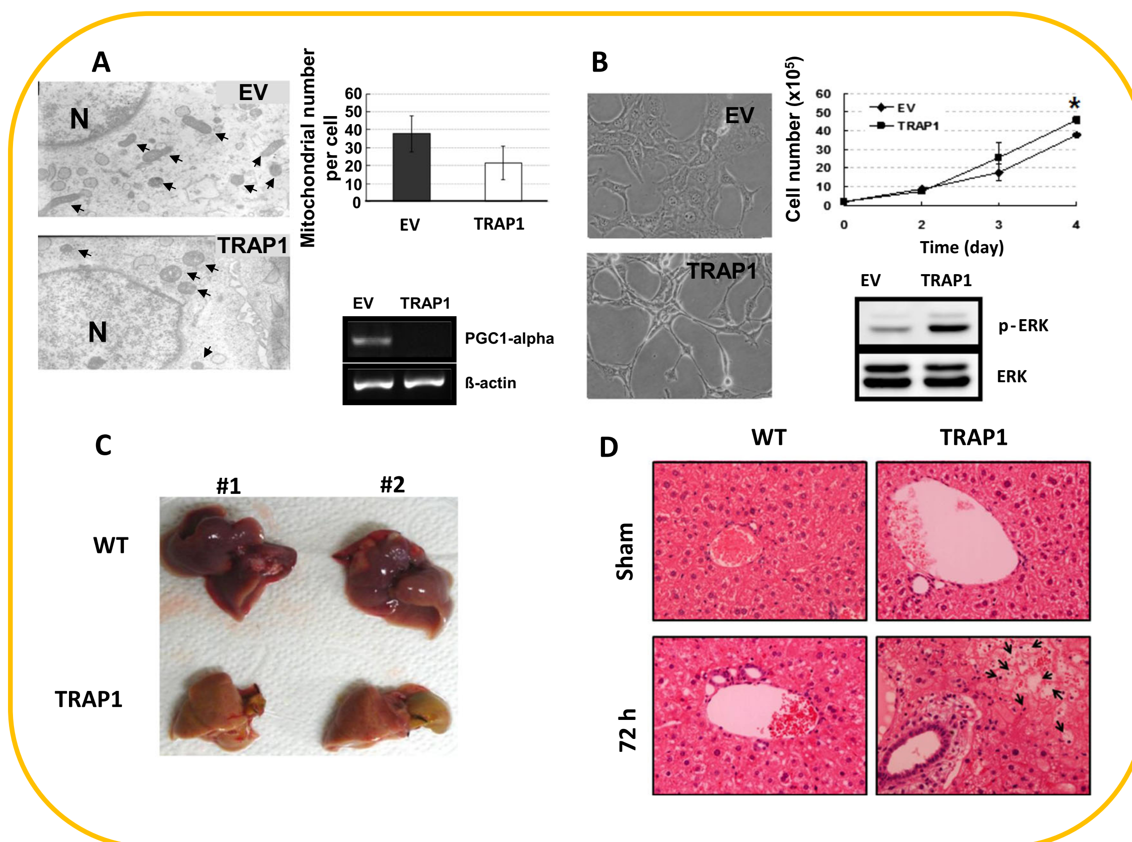

Fig. 2 Effects of TRAP1 overexpression on in vitro and in vivo models. **a** Mitochondrial morphology by transmission electron microscopy illustrating decreased mitochondrial numbers in stable TRAP1-expressing cell lines (*upper panel*). Decreased PGC-1α mRNA levels in TRAP1 cells (*lower panel*). **b** Altered morphology, enhanced proliferation, and ERK phosphorylation in TRAP1 cells. **c** Fat accumulation in the liver at 72 h after partial hepatectomy (PH). **d** Pathological analysis of liver tissues at 72 h after PH in wild-type (WT) and TRAP1 transgenic mice; hematoxylin and eosin (H&E) staining. (**a**, **b** reprinted by permission from Im and Seo (2014); **c**, **d** from Im et al. (2013)

dynamics and function with tumorigenesis in breast cancer (Zhang et al. 2015a).

Neurodegenerative disorders: PD

Parkinson's disease (PD) is a degenerative disorder of the central nervous system mainly affecting the motor system. The motor symptoms of PD result from the death of dopamine-generating cells in the substantia nigra, but the causes of this cell death are poorly understood. The most obvious symptoms are movement-related, including shaking, rigidity, slowness of movement, and difficulty with walking and gait. Later, cognitive and behavioral problems may arise, with dementia commonly occurring in the advanced stages of the disease; depression is the most common psychiatric symptom. Other symptoms include sensory, sleep, and emotional problems. PD is more common in older people, with most cases occurring after the age of 50 years. The genetic etiology of PD is associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes (Nuytemans et al. 2010). Among them, pivotal roles for PINK1 and autophagy in mitochondrial quality control have been implicated in PD (Chu 2010). PINK1 is a mitochondrial serine/threonine kinase. TRAP1 has been shown to interact with PINK1 affecting PD. Pridgeon et al. identified TRAP1 as a cellular substrate for PINK1, which interacts and colocalizes with TRAP1 in the mitochondria, to phosphorylate it. The ability of PINK1 to promote both TRAP1 phosphorylation and cell survival is impaired by the PD-associated PINK1 mutations G309D, L347P, and W437X, indicating that PINK1 is upstream of TRAP1 and mutation of PINK1 might affect TRAP1 function. The ability of PINK1 to protect cells against oxidative stress depends on its kinase activity (Pridgeon et al. 2007). Drosophila TRAP1 also protects against mitochondrial dysfunction in a PINK1 model of PD (Costa et al. 2013). As shown in Fig. 1, loss of TRAP1 results in decreased mitochondrial function and increased sensitivity to stress (paraquat, rotenone, and antimycin) and its upregulation in neurons of PINK1 mutants rescues mitochondrial impairment. Moreover, the expression of TRAP1 partially rescued mitochondrial impairment in PARKIN mutant flies, and conversely, expression of PARKIN rescued mitochondrial impairment in TRAP1

mutants, indicating that TRAP1 works downstream of PINK1 and in parallel with PARKIN in Drosophila. In another study, however, Zhang et al. reported that overexpression of human TRAP1 was able to mitigate PINK1 but not PARKIN loss-of-function phenotypes in Drosophila. In human neuronal SH-SY5Y cells, TRAP1 also rescued mitochondrial fragmentation and dysfunction after siRNA-induced silencing of PINK1 but not PARKIN (Zhang et al. 2013). This discrepancy in the relationships between PARKIN and TRAP1 remains poorly understood. Recently, Fallaize et al. found that PINK1 resides in the cristae membrane and intracristae space but not on the outer mitochondrial membrane (OMM) of healthy mitochondria. This localization seems to be altered by stimuli. Under normal physiological conditions, PINK1 is colocalized with its substrate TRAP1 in the cristae membrane and intracristae space. In response to mitochondrial depolarization, PINK1, but not TRAP1, translocates to the OMM, suggesting that differential submitochondrial localization of PINK1 serves as a molecular switch mediating two distinct mitochondrial signaling pathways to maintain mitochondrial homeostasis (Fallaize et al. 2015). Following this observation, it may be informative to define whether mutations in PINK1 affect the function of TRAP1 in stress-induced neuronal death.

Metabolism

Cells acquire energy in the form of ATP via two main pathways: glycolytic conversion of glucose into pyruvate in the cytosol and the tricarboxylic acid (TCA) cycle in mitochondria. Yoshida et al. first explored the metabolic and phenotypic consequences of TRAP1 gene disruption/knockdown or overexpression in fibroblast cell lines established from adult WT and TRAP1-null mice and in human tumor cells transiently transfected with either TRAP1-specific siRNA or TRAP1 expression plasmids. They demonstrated that loss of TRAP1 increased mitochondrial oxygen consumption, elevated levels of TCA cycle intermediates, and increased ATP and ROS levels, with concomitant suppression of aerobic glycolysis, together with strikingly enhanced invasiveness. Overexpression of TRAP1 has the opposite effect. Loss of c-Src expression abrogates the ability of TRAP1 to modulate mitochondrial respiration and ATP levels, and TRAP1 and c-Src colocalize and interact within mitochondria. Interestingly, TRAP1 expression is also inversely correlated with tumor grade in several cancers. The findings of the Yoshida group suggest that TRAP1 acts as a tumor suppressor (Yoshida et al. 2013). This is in contrast to current opinion that TRAP1 functions as an oncoprotein, based on its elevated expression in various cancers and on our previous finding that overexpression of TRAP1 leads to increased basal ROS levels and enhanced proliferation (Im and Seo 2014). This discrepancy may arise from cell-type-specific and/or gene expression methodology differences among studies and requires further clarification.

Meanwhile, in another study, we established TRAP1 transgenic mice and found that TRAP1 overexpression led to fatty liver and increased inflammation after partial hepatectomy (PH) (Im et al. 2013). The incidence of fatty liver in TRAP1 transgenic mice was significantly higher (~50 %) than that in WT control mice (0 %) at 48 and 72 h after PH (Figs. 1 and 2). Although the mechanism remains unknown, these findings suggest that TRAP1 plays a critical role in balancing energy substrates during liver regeneration by regulating lipid accumulation. The most common liver pathology is nonalcoholic fatty liver disease (NAFLD), which is characterized by intrahepatic accumulation of lipids. NAFLD can evolve into nonalcoholic steatohepatitis (NASH) in the presence of oxidative stress and inflammation, and NASH is a serious risk factor for liver diseases such as cirrhosis and hepatocellular carcinoma. Among the patients who develop NASH, up to 20 % may advance to cirrhosis and are at risk for complications of end-stage liver disease. One of the major complications observed in patients with NASH-related cirrhosis is hepatocellular carcinoma (HCC), which has emerged as the sixth most common cancer and second leading cause of cancer-related deaths worldwide (Khan et al. 2015). Little is known about the role of TRAP1 in the liver, but it is highly expressed in normal liver and has been suggested as a novel biomarker for HCC (Megger et al. 2013). TRAP1 has been found to dampen activation of the nutrient-sensing AMP-activated protein kinase (AMPK) and to overcome metabolic stress and promote tumor cell metastasis by limiting the ability of AMPK to trigger autophagy (Caino et al. 2013), indicating that TRAP1 is involved in AMPK-related energy sensing. Therefore, elucidation of the role of TRAP1 in the liver may contribute significantly to the prevention of metabolic diseases, including HCC, and to providing therapeutic strategies. In addition, TRAP1 transgenic mice may be a good in vivo model to study its role in metabolic regulation such as AMPK activation and how its malfunction may affect carcinogenesis (Fig. 1).

Emerging role: stemness in CSCs

Stem cells have self-renewal activity and the ability to differentiate into multiple cell types during development and tissue repair after damage, maintaining cellular and tissue homeostasis. Unfortunately, the roles for TRAP1 in stem cells during development remain undefined. Involvement of the TRAP-1 homologue Dd-TRAP1 in spore differentiation during *Dictyostelium* development has been reported (Morita et al. 2005). Dd-TRAP1 synthesized at the vegetative growth phase is retained during the entire course of *Dictyostelium discoideum* development; at the multicellular slug stage, it is located in prespore-specific vacuoles (PSVs) of prespore cells,

as well as in the cell membrane and mitochondria. Dd-TRAP1 in PSVs was exocytosed during sporulation to constitute the outermost layer of the spore cell wall. In RNAi-mediated TRAP1-silenced cells, PSV formation and, therefore, prespore differentiation were impaired significantly, particularly under heat-stress conditions. This suggested that Dd-TRAP1 might be involved in late development, including spore differentiation, as well as in early development.

Tumors comprise heterogeneous groups of cells, in which a specific subpopulation of cancer stem cells (CSCs) is proposed to be responsible for tumorigenesis, resistance to therapy, and recurrence, leading to metastasis from primary sites to distant organs via epithelial-to-mesenchymal transition (EMT) (Kapoor and Kumar 2014). This behavior has been attributed to the extensive self-renewal and multipotent differentiation abilities of CSCs, characteristics similar to those of other stem cells (Jordan et al. 2006). Specifically, CSCs express a signature series of proteins, including SOX-2, OCT-4, and CD133 (Chen et al. 2007; Sahlberg et al. 2014; Tam and Ng 2014); in vitro, they display enhanced levels of several stemness-related transcription factors, such as SOX-2, under specific culture conditions (Im et al. 2015).

Several lines of evidence suggest that TRAP1 may be involved in the maintenance or regulation of stemness in CSCs; while CSCs are a small subpopulation within tumors, HSP90 family members, including TRAP1, are associated with tumorigenesis. First, several reports have demonstrated that TRAP1 is associated with drug resistance, migration, and/or invasion (Matassa et al. 2014; Agliarulo et al. 2015; Wu et al. 2015; Zhang et al. 2015a), in some colon and ovarian cancers (Costantino et al. 2009; Landriscina et al. 2010a; Maddalena et al. 2013), a characteristic of CSCs. Second, most tumors experience severe hypoxia and adapt to these harsh conditions via mitochondrial alterations. TRAP1 is a mitochondrial HSP that is highly expressed in many tumors and cancer cells. Third, TRAP1 transgenic mice and TRAP1-overexpressing cells display both aberrant mitochondria and mitochondrial dysfunction (Im et al. 2007, 2013; Im and Seo 2014). Recently, Kadye et al. hypothesized that TRAP1 serves to modulate mitochondrial activity in stem cell maintenance (Kadye et al. 2014), although evidence for this remains elusive. In agreement with that hypothesis, Lettini et al. reported that TRAP1 represents a key mediator of stemness and glycolytic metabolism in colorectal cancer cells (Lettini et al. 2014). A recent paper also provides important evidence that TRAP1 is involved in the regulation of CSCs. Wu et al. demonstrated that TRAP1 is crucial for the Warburg effect (Alfarouk et al. 2014) in human glioblastoma multiforme (GBM). In contrast to normal brain, TRAP1 is highly expressed in GBM. TRAP1 depletion strongly decreased GBM cell proliferation and migration, inhibited neurosphere recovery, secondary neurosphere formation, and enhanced the therapeutic effect of temozolomide in neurosphere cultures. Since cancer cells preferentially use aerobic glycolysis to support growth, a metabolic alteration commonly referred to as the "Warburg effect, " downregulation of TRAP1 seems to inhibit tumor growth and migration through its regulatory effects on metabolic reprogramming in GBM (Wu et al. 2015). In addition, Yoshida et al. also demonstrated that TRAP1 regulates a metabolic switch between mitochondrial respiration and aerobic glycolysis (Yoshida et al. 2013). Considering that Warburg effect is a crucial metabolic switch and a metabolic reprogramming of CSCs is considered as another cancer hallmark (Menendez et al. 2013), TRAP1 might contribute to this progress (Fig. 3). Although TRAP1 associated with tumor specimens is clear, its involvement in the initiation of tumors is not yet convincing. Hence, it will be interesting to determine whether TRAP1 knockdown or its overexpression affects stemness-related genes such as SOX-2 and/or sphere-forming activity, which are representative properties of CSCs. Furthermore, since CSCs display metastasis and drug resistance, it will also be important to evaluate whether manipulating TRAP1 levels alters these properties. In parallel, it is an open question as to whether TRAP1-positive or TRAP1-negative cells in general display stemness properties as described above. Hence, future studies should address the role of TRAP1 in stemness of other types of stem cells, as well as CSCs.

Inhibitors targeting TRAP1

As described above, mitochondrial TRAP1, like HSP90 in the cytoplasm, is highly expressed in various cancers. Several studies have examined organelle-specific inhibitors of targets including HSP90 (Seo 2015). Considering that mitochondria play crucial roles in structural integrity and oxidative stress, as well as in apoptosis, it is reasonable that TRAP1 is a good target for inducing apoptosis in cancer. As shown in Fig. 1, Tsutsumi et al. first identified dimethylaminoethylamino-17-demethoxygeldanamycin (DMAG)-N-oxide as a cell-impermeable HSP90 inhibitor, after analyzing a number of geldanamycin-derived molecules for membrane permeability and binding affinity to HSP90 (Tsutsumi et al. 2008); this inhibitor may act on both HSP90 and TRAP1 since several reports suggested extracellular HSP90 and TRAP1 localization. For instance, immunoelectron microscopy revealed that TRAP1 localizes at specific extramitochondrial sites such as the cell surface of blood vessel endothelial cells (Cechetto and Gupta 2000). Based those observations, it will be intriguing elucidation of their roles and targeting them in extracellular matrix (Seo 2015). Kang et al. designed

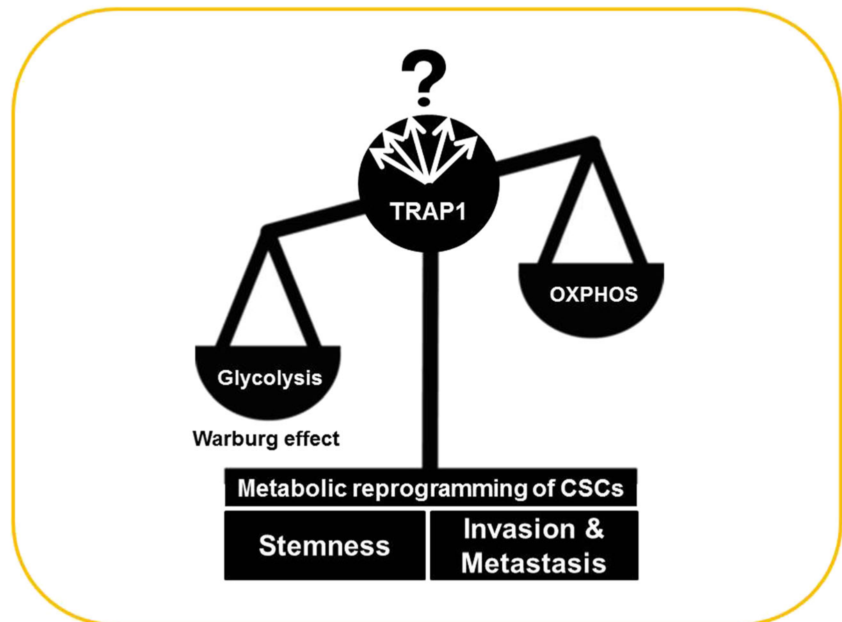

Fig. 3 TRAP1 as a switch from oxidative metabolism (oxidative phosphorylation, OXPHOS) to glycolysis in metabolic reprogramming of CSCs. TRAP1 levels alter mitochondrial respiration and fatty acid oxidation, together with cellular accumulation of tricarboxylic acid cycle intermediates, ATP, and ROS. At the same time, TRAP1 deficiency affects glucose metabolism as well as invasiveness (Yoshida et al. 2013)

gamitrinibs, mitochondria-targeted small molecules that inhibit both TRAP1 and HSP90 inside the mitochondria (Kang et al. 2009; Kang 2012) (Fig. 1). Conventional HSP90 inhibitors do not affect the function of mitochondrial HSP90 or TRAP1 directly, nor do they induce mitochondrial dysfunction, probably owing to their inability to penetrate the mitochondrial membrane. However, gamitrinibs inhibit mitochondrial HSP90 and TRAP1 function directly. TRAP1 has been shown to play a critical role in regulating the opening of the mitochondrial transition pore induced by excessive ROS production. Kang et al. demonstrated that gamitrinibs promoted activation of the peptidyl-prolyl cis-trans isomerase CypD, opening of the mitochondrial permeability transition pore, release of cytochrome c, and induction of cell death in cancer cells. Although TRAP1 and HSP90 seem to work independently and be functionally redundant in mitochondria, use of current inhibitors containing gamitrinibs has not been successful in distinguishing the two. Targeting multiple proteins that are overexpressed in various cancer cells, such as HSPs and TRAP1, may be another strategy for cancer therapy; while not specific, it can be effective, as has been seen with GTEs (Zhang et al. 2011). Lee et al. tried to develop mitochondrial TRAP1 inhibitors and replaced the isopropyl amine of the Hsp90 inhibitor PU-H71 with the mitochondria-targeting moiety triphenylphosphonium to produce SMTIN-P01, which showed TRAP1-specific activity and had improved cytotoxicity to cancer cells (Lee et al. 2015). Recent advances in delivery systems that have combined specific polymers and siRNA (An et al. 2015) suggest that it will be possible to specifically target TRAP1 by this method. In the near future, it should also be helpful to utilize organoids (Gjorevski et al. 2014; Ranga et al. 2014) in vitro and an avatar mouse model (Ohman et al. 2014) in vivo to explore the therapeutic potential of TRAP1 inhibitors for diseases such as cancer and PD.

Conclusions and perspectives

Mitochondrial TRAP1 plays important roles in regulating mitochondrial integrity, protecting against oxidative stress, and inhibiting cell death. Hence, pharmacological activation or inactivation of TRAP1 will alter mitochondrial function and concomitant cellular fate selectively in aging, unhealthy, or cancer cells, including CSCs, suggesting that it may be a good therapeutic target. A small number of studies have attempted to determine the mechanism of action of TRAP1 (Masuda et al. 2004). Several drug candidates targeting TRAP1 have been developed that have shown strong cytotoxic activity against many cancers, but not against normal cells, in vitro and in vivo (Kang 2012). However, they are only able to inhibit TRAP1; further development would be required to induce dysfunctional TRAP1 to function normally. Elucidation of TRAP1 functions in a variety of processes should guide new approaches to TRAP1 modulation for therapy. The establishment of appropriate model systems in vitro and in vivo is also necessary to elucidate the mechanisms and develop specific inhibitors.

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