TRAP1 is involved in ER stress protection of cancer cells

TRAP1 (Tumour Necrosis Factor Receptor-Associated Protein 1) is a molecular chaperone, member of the HSP90 family, that contributes to the overall survival of cancer cells and is up-regulated in most tumour types (reviewed in 1). A large body of literature demonstrates that TRAP1 is part of a pro-survival signalling pathway aimed at evading the toxic effects of oxidants and anticancer drugs and plays a role in protecting mitochondria against apoptotic stimuli (reviewed in 2). However, whether its roles are uniformly oncogenic or not is now a matter of intense debate. Interestingly, Yoshida and colleagues [3] propose a more subtle reading of TRAP1 roles in the regulation of cellular metabolism and its impact on tumorigenesis. In fact, an inverse correlation between TRAP1 expression and tumor stage in cervical, bladder, and clear cell renal cell carcinoma was demonstrated. These conflicting observations on TRAP1 “behaviour” in different biological contexts is strictly related to a specific molecular complex/integrated network in which TRAP1 represents one of the focal nodes.

Abbreviations

BC - breast cancer;
CRC - colorectal carcinoma;
ER - endoplasmic reticulum;
HSP - heat shock protein;
IRES - internal ribosome entry site;
KD - knockdown;
MTP - Mitochondrial Transition Pore;
shRNA - short-hairpin RNA;
siRNA - small interfering RNA;
S6K - S6 kinases;
TRAP1 - TNF receptor-associated protein 1;
UPR - unfolded protein response.

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In this scenario, our recent evidence demonstrating that TRAP1 is involved in the crosstalk between mitochondria and Endoplasmic Reticulum (ER) and in ER stress protection of tumor cells appear remarkable: in fact, our group, for the first time, reported a role of TRAP1 in protein quality control, due to its interaction with the proteasomal particle TBP7 outside of mitochondria, namely on the outer side of the ER [4]. Given the ER localization of TRAP1 and TBP7, it became important to assess the involvement of these two proteins in ER homeostasis. Consistently, we demonstrated that, upon stress induction, colorectal carcinoma (CRC) cells, in which the expression of TRAP1 was stably knocked down by sh-RNAs, are characterized by an increased expression of BiP/Grp78, the major ER chaperone essential for protein quality control in the ER [5] and a marker of ER stress conditions. Accordingly, this phenotype is rescued upon TRAP1 re-addition and/or transfection of a TRAP1 deletion mutant (Δ1-59-TRAP1), which lacks the mitochondrial targeting sequence and is therefore unable to enter mitochondria; conversely, another deletion mutant with mitochondrial localization (Δ101-221-TRAP1) not only is unable to counteract ER stress, but even further increased BiP/Grp78 levels. The interference of either TRAP1 or TBP7 proteins results in the induction of apoptosis both in response to drug-induced ER stress and in increased intracellular protein ubiquitination, which is selectively rescued by the re-addition of TRAP1 [4]. The elimination of misfolded proteins represents an important mechanism to maintain cell viability. Interestingly, disorders of protein folding and degradation are emerging as fundamental mechanisms in the pathogenesis of many diseases, in particular tumor progression [6].

Accordingly, similar evidences were obtained studying human breast carcinoma (BC), another tumor cell model characterized by up-regulation of TRAP1. Among 23 BCs characterized for both TRAP1 and BiP/Grp78 expression, a significant co-upregulation of the two genes was observed [7]. These data suggest that UPR is activated in a significant subgroup of human BCs, as a result of chronic exposure to ER stress conditions, and that, remarkably, TRAP1 is part of this cell response to ER stress.

Adaptation to ER stress conditions represents a mechanism of resistance to taxanes [8-10]; interestingly, our data show that paclitaxel induces apoptotic cell death and caspase 12 activation in MCF7 BC cells and enhances BiP/Grp78 and Grp94 expression [7]. Also in this context, a role of TRAP1 has been demonstrated by our groups: in fact, paclitaxel induces TRAP1 protein levels in MCF7 and MDA-MB231 BC cells, without affecting its mRNA expression. TRAP1 knockdown (KD) increases apoptosis upon paclitaxel treatment in both BC cell lines, whereas TRAP1 re-expression in TRAP1 KD cells resulted in significant protecting activity. Analysis of the UPR response in TRAP1 KD MCF7 cells showed reduced BiP/Grp78 levels compared to control cells, in agreement with the co-expression of TRAP1 and BiP/Grp78 in human BCs. By contrast, paclitaxel increased BiP/Grp78 and Grp94 expression levels and caspase 12 cleaved product in TRAP1 KD cells compared to controls. Moreover, ER-associated TRAP1 modulates the activation of stress pathways responsible for regulation of protein synthesis rate and survival responses: PERK and eIF2α are in fact efficiently phosphorylated in control MCF7 cells upon paclitaxel exposure, whereas down-regulation of TRAP1 resulted in reduced activation of the PERK/eIF2α pathway. Interestingly, in addition to being insensitive to paclitaxel, paclitaxel-resistant cells exhibited resistance to other agents inducing ER stress (i.e., thapsigargin and bortezomib), whereas TRAP1 down-regulation by siRNA re-established sensitivity to both paclitaxel and ER stress inducers [7]. These data suggest that adaptation to paclitaxel and ER stress share common molecular mechanisms and that TRAP1 plays a role in taxane resistance through ER stress protection. We observed significant cytoprotective activity toward these agents due to re-expression of both wild type TRAP1 and the mitochondrial import-defective TRAP1 mutant, confirming the relevant role of TRAP1 in the protection from ER stress and in protein quality control, and that this function, exerted from ER-associated TRAP1, is relevant for its antiapoptotic activity. Consistently, the combination of paclitaxel with sub-cytotoxic concentrations of Gamitrinib, that targets the mitochondria-resident TRAP1 only, resulted in additive apoptotic effects in BC MCF7 cells as well as only partially reverting resistance to this agent in paclitaxel-resistant MCF7 cells [7].

ER-associated TRAP1 also favours resistance to anthracyclins, which also induce ER stress in BC cells; analogously, cross-resistance between ER stress agents and anthracyclins was observed in bortezomib- and anthracyclin-resistant cells [11].

2 TRAP1 confers resistance to ER stress through a modulation of stress-responsive protein’s expression

eIF2α phosphorylation is the first-line response to ER stress and allows the immediate attenuation of global
protein synthesis and selective translation of stress-responsive genes, to allow a rapid cell response to changes in extracellular environments [12]. Of note, TRAP1-containing HCT116 cells exhibit higher activation of the eIF2α kinase PERK and, consequently, higher amounts of phospho-eIF2α either before or after ER stress induced by thapsigargin. By contrast, shTRAP1 cells fail to activate eIF2α in response to stress conditions [13]. The ability of TRAP1 to modulate eIF2α phosphorylation is particularly relevant for its cytoprotective properties, since it has been demonstrated that eIF2α phosphorylation induces the preferential translation of a group of stress-responsive mRNAs, including the transcription factor ATF4; accordingly, higher levels of ATF4 were detected in scrambled cells upon amino acid or glucose deprivation. Moreover, preferential activation of eIF2α upregulates ATF4-target genes involved in amino acid synthesis and transport [14], as well as in response to oxidative or ER stress itself, and, among others, xCT, the specific subunit of cystine/glutamate antiporter system [15], whose regulation could contribute to the well characterized role of TRAP1 in oxidative stress protection, and BiP/Grp78. Consistently, BiP/Grp78 mRNA expression is decreased in TRAP1 KD cells under basal conditions [13]. Indeed, as stated above, cells expressing high levels of TRAP1 show reduced sensitivity to ER stress inducers, whereas TRAP1 KD cells respond with a much higher compensatory upregulation of BiP/Grp78 under treatment.

Another protein involved in ER stress protection, which is relevant in TRAP1 pathway, is Sorcin, a Ca2+-binding protein widely distributed among mammalian tissues [16]. Sorcin behaves as a Ca2+-sensor, being involved in the regulation of Ca2+ homeostasis and excitation-contraction coupling in the heart [17,18]. Several lines of evidence suggest that Sorcin is up-regulated in human malignancies, where it is involved in cytoprotective functions and drug resistance. The potential involvement of Sorcin in cytoprotection is consistent with the evidence that the isoform B, whose expression is restricted to mitochondria, is a TRAP1-binding protein [4,19]. Remarkably, TRAP1 regulates, together with TBP7, the ubiquitination of Sorcin isoform B; consequently, Sorcin expression levels are decreased upon TRAP1 interference, as a consequence of increased ubiquitination [4]. The 22kDa isoform of Sorcin, which is the most abundant cellular isoform and is up-regulated in about 50% of human CRCs, is not a TRAP1 interacting protein [19]; however, in human CRCs, TRAP1 expression significantly correlates with the protein levels of 22kDa Sorcin [20]. Indeed, 22kDa Sorcin is an ER-resident protein up-regulated in conditions of ER stress and responsible for enhancing the accumulation of Ca2+ in the ER, thus preventing ER stress. Its silencing favors the activation of caspase-12 and Grp78/BiP in response to stress and induces apoptosis through the mitochondrial pathway [20]. Based on such evidence, it is reasonable to hypothesize that both Sorcin isoforms are involved in regulating Ca2+ homeostasis in separate cell compartments and that this function is relevant for their antiapoptotic activities in tumor cells. While the 22kDa Sorcin isoform is one of the several ER stress proteins involved in the control of Ca2+ levels in the ER, preventing ER stress and subsequent apoptotic events, it is likely that Sorcin isoform B is critical in controlling Ca2+ homeostasis in mitochondria, thus contributing to the regulation of the opening of the mitochondrial transition pore (MTP). This hypothesis sheds some light on the relevance of the TRAP1-Sorcin interaction and the role of the mitochondrial Sorcin isoform in TRAP1 cytoprotective pathway. Indeed, TRAP1 activity is crucial for the expression/ubiquitination of such Sorcin isoform [13], potentially contributing to Sorcin-induced regulation of Ca2+ homeostasis in mitochondria and participating in the Ca2+-dependent MTP regulation. This observation may be relevant in the perspective of evaluating TRAP1 network as a potential molecular target to revert drug resistance. Indeed, the modulation of Ca2+ homeostasis is a crucial step in the cell response to stress conditions and in favoring the multidrug resistance phenotype in human tumors [21]. It is intriguing to speculate that TRAP1 and Sorcin are both involved in the cross-talk between mitochondria and ER stress response pathways, being components of a coordinated adaptive response of tumor cells to counteract ER stress conditions and apoptotic signaling. In support of this view, our group has shown that the mitochondrial Sorcin upregulation is able to protect MCF7 cells against paclitaxel-induced apoptosis. In support of this inter-organelles communication model, Siegelin et al. [22] found that impairing HSP90s functions within mitochondria of tumor cells by Gamitrinib results in upregulation of BiP/Grp78 and phosphorylation of eIF2α. Accordingly, Takemoto et al. [23] reported that PERK activation levels decreased in the TRAP1 KD cells in comparison with those of control cells, with corresponding low level of eIF2α phosphorylation. In parallel, TRAP1 KD showed compensatory induction of BiP/Grp78 expression by increasing cleavage level of ATF6, likely as a mechanism to protect them from ER stress-induced cell death. These data candidate TRAP1 as one of the key molecules in the signaling pathway involved not only in mitochondrial stress, but also in ER stress-induced apoptosis, although they are unable to clarify the molecular mechanisms by which TRAP1 influences the ER stress response.
3 TRAP1 regulation of tumour cell metabolism is based on attenuation mechanisms

As solid cancers grow, their nutrient requirements eventually exceed the capacity of the existing vascular bed. Although many cancers adapt by triggering angiogenesis, inevitably the cores of most tumors become hypoxic and nutrient depleted. Impaired generation of ATP compromises ER protein folding, thus leading to activation of the UPR. Indeed, phosphorylation of eIF2α by PERK has been shown to be necessary for the growth of larger solid tumors [24]. As reported above, the role of TRAP1 in favouring eIF2α phosphorylation in cancer cells is well characterized [13,25]. Using several inducible Myc cell models, as well as genetic and pharmacologic tools, Hart et al. [26] have recently shown that Myc induction leads to activation of the PERK/eIF2α/ATF4 axis of the UPR, resulting in increased autophagy and protection against ER stress-dependent apoptosis. Similarly, we have demonstrated in CRC that increased TRAP1 expression correlates with increased expression of Myc, Grp78/BiP, ATF4 and increased phosphorylation of eIF2α [7,13,25].

The increased protein synthesis and oxidative stress are necessary signals for ER stress-induced cell death [27]. Molecular chaperones are often bound to newly synthesized polypeptides [28], assisting their folding while they are still bound to ribosomes [29]. Little is known about chaperones assisting protein folding in humans, analogously to the yeast chaperones linked to protein synthesis (CLIPS), which are distinct from the stress-induced chaperones [30].

Duttler et al. [31], measuring ribosome-associated ubiquitination in budding yeast, found that, under normal growth conditions, approximately 5% of newly made polypeptides are ubiquitinated. Using an in vitro mammalian system, another group reported that 12–15% of nascent polypeptides are ubiquitinated [32]. Both groups report that a reduction of the ribosome-associated chaperones yields increased co-translational ubiquitination. Interestingly, we have demonstrated that TRAP1 acts as a regulator of both protein synthesis and degradation, by reducing co-translational ubiquitination in concert with the proteasomal particle TBP7 [13], most likely by mediating the cross-talk between translation

Figure 1: TRAP1 protects cells from ER stress by regulating protein homeostasis. On the outer side of the ER, TRAP1 is associated with both the protein synthesis machinery (ribosomes and translation factors) and the proteasome, thus regulating the rate of protein synthesis and the ubiquitin-dependent degradation of specific substrates, such as the mitochondrial FIATPase and Sorcin isoform B. Consequently, the global protein synthesis is attenuated, whereas the IRES-dependent translation is enhanced, with consequent upregulation of stress-responsive proteins such as BiP. Therefore, TRAP1 expressing tumor cells are able to promptly respond to ER stress or nutrient deprivation, respectively through the PERK or the GCN2 pathway, simultaneously mediating cytoprotective pathways inside mitochondria.
elongation, translation initiation and co-translational protein quality control [25]. Our experimental model so far proposes that TRAP1 is indeed associated with active polyribosomes and several translation factor, ultimately attenuating protein translation to maintain proteostasis (Figure 1).

Recent work reported that newly synthesized polypeptides are more sensitive than older polypeptides to proteotoxic stress, and they are selectively degraded by the ubiquitin-proteasome machinery [33]. It is not surprising, therefore, that, in addition to induction of chaperones through the heat shock transcription factor Hsf1, cells often rely on translational control for an immediate and rapid response to stress [34]. Indeed, global protein synthesis is reduced in response to many types of adverse conditions, which limits the load of the protein quality control system through decreased protein production [35]. Current models for the mechanism governing this translational attenuation are largely limited to initiation regulation. However, recent studies demonstrate that translation elongation is also involved in the phenomenon. Using a genome-wide ribosome profiling approach, two groups independently reported widespread pausing of ribosomes early in elongation in response to proteotoxic and heat shock stress [36,37]. Because ribosome-associated chaperone molecules are located near the exit of the tunnel, it is conceivable that translation elongation is also influenced by chaperone availability. However, it is still unclear mechanistically how the absence of chaperones brings translation to a halt. Noteworthy, TRAP1 directly binds eEF1A, which, in turn, is able to repress the activity of the eEF2α kinase, GCN2 [38]. We have hypothesized that TRAP1 control on GCN2-mediated eEF2α phosphorylation might involve eEF1A-GCN2 interaction. Consistently, eEF2α phosphorylation is decreased upon eEF1A transfection only in high TRAP1 background, demonstrating that cells expressing high levels of TRAP1 are able to modulate the inhibitory effect of eEF1A towards GCN2 [25].

The tight connection between the regulation of protein synthesis initiation and elongation is confirmed by the evidence that two well-characterized regulators of translation are the eukaryotic initiation factor 4E binding proteins (4E-BPs) and the p70 ribosomal S6 kinases (S6Ks) [39,40]. 4E-BPs are the major effectors of mTORC1 in controlling cap-dependent translation initiation, whereas S6Ks act on multiple stages including elongation through eEF2K. Notably, the phosphorylation of S6Ks is rapamycin-sensitive, whereas the phosphorylation of 4E-BPs is largely rapamycin-resistant [41]. Indeed, rapamycin rescues the quality of translational products mainly by slowing down the rate of ribosomal elongation. The most striking demonstration of TRAP1 regulation of protein translation arises from the observation that expression and consequent phosphorylation of p70S6K is increased upon TRAP1 silencing [25]. Consistently, TRAP1 KD cells show increased incorporation of radiolabeled amino acids [13], increased levels of active polysomes and increased sensitivity to translational stress and drugs targeting protein synthesis [25]. Interestingly, TRAP1-expressing cells show higher ratio between IRES- and cap-dependent translation, consistent with their higher eEF2α phosphorylation levels [25]. This mechanism is relevant in cancer development, because among 70 experimentally verified cellular IRES elements [42], a large number are found in cancer-related genes [43].

In a recent study, Leprivier et al. [44] found that the adaptation of tumor cells to nutrient deprivation is dependent on their ability to acutely block translation elongation during protein synthesis through a control of eEF2 activity. Increased eEF2K levels found in tumors might promote tumor cell survival, by providing protection from nutrient deprivation. Reciprocally, eEF2K null tumors displayed increased necrosis and apoptosis under calories restriction. While the dependence on this mechanism under nutrient-depletions is not specific for tumor cells, it might be particularly important in the context of the tumor microenvironment, which would make pharmacological inhibitors to eEF2K, alone or in combination with other compounds, effective cancer treatments.

Several studies both with in vitro translation systems and cell cultures indicated that high rates of translation reduce fidelity and promote misincorporation of amino acids [45], which could lead to protein misfolding. Reduced elongation speed, by contrast, favors the correct tRNA pairing. It is thus conceivable that an increased translation speed generates more aberrant translational products. In fact, it was reported that inhibition of translation by only 15-20% strongly reduced accumulation of ubiquitinated species upon proteasome inhibition, and almost completely prevented formation of an agglomerate of protein aggregates, the aggresome [46,47], which is mediated by eEF1A, a TRAP1 binding partner [13]. Consistent with this model, eEF1A has also been involved in cotranslational ubiquitination [48]. Notably, eEF1A and TBP7, both TRAP1 client proteins, have been found in complex with the translation initiation factor IF3, taking part in the so-called “translasome”, in which factors involved in translation initiation, ribosome biogenesis, translation elongation, quality control, and transport are physically linked to facilitate efficient protein synthesis [49].
4 The hypothesis of the integrated control by TRAP1: hit ER stress by targeting the net

TRAP1-dependent regulation of protein synthesis provides cancer cells with enhanced capabilities to survive nutrient deprivation and translational stress [13]. This represents a remarkable example of how cancer genes function as network of hub proteins involved in multiple cellular processes and form focal nodes in the exchange of information between diverse signaling pathways. These metabolic adaptations must balance three crucial requirements of tumor cells: increased energy production, sufficient macromolecular biosynthesis and maintenance of redox balance. It has already been demonstrated that TRAP1 stays at the crossroad of these three processes. In fact, i) TRAP1 contributes to the tumor’s switch to aerobic glycolysis through inhibition of succinate dehydrogenase, the complex II of the mitochondrial respiratory chain [3,50]; ii) TRAP1 is part of a pro-survival signalling pathway aimed at evading the toxic effects of oxidants and anticancer drugs, and this effect is mediated by its capacity to protect mitochondria against damaging stimuli via a decrease of ROS generation (reviewed 51); iii) TRAP1 controls protein homeostasis through a direct involvement in the regulation of protein synthesis and co-translational protein degradation [13]. Recently, striking evidence suggests that transcription and translation can also be coupled in eukaryotes through the “remote controlling” of translation by the transcription apparatus, namely by Rpb4p and Rpb7p proteins, two components of RNA polymerase II [52]. Preliminary data show that TRAP1 co-sediments with translationally active polyribosomal particles and that TRAP1 KD cells have higher levels of protein synthesis. Public databases, based on bioinformatic predictions, report the presence in TRAP1 structure of a Ribosomal S5 D2-type fold domain, often found in numerous RNA/DNA-binding proteins, including translational machinery components. Moreover, TRAP1 associates with 3’UTR region of the mRNA for the mitochondrial ribosomal protein S12 [53]. It can be hypothesized that TRAP1-dependent regulation of translation represents one of the focal nodes linking different parts of the regulatory network.

Recent studies on the integration of ER stress signaling pathways with metabolic stress, oxidative stress, and inflammatory response signaling pathways provide new insights into the diverse cellular processes that are regulated by the UPR [54]. In such a scenario, a new wave of small-molecule anticancer agents is emerging, targeting complex multicomponent cellular machineries, which interfere with integrated molecular systems selectively activated by cancer cells, and likely less relevant for normal cells, due to chronic stress conditions and the unfavourable microenvironment experienced by tumors. HSP90s in general have long been considered targets of interest for the treatment of cancers, since they are molecular chaperones required for the folding and stability of many oncogenic signaling proteins and inhibiting proteins that regulates multiple signal transduction pathways in cancer cells is an attractive approach for cancer therapy. However, several HSP90 family member inhibitors failed to enter the clinical setting because of severe toxicities, likely due to their unspecific inhibition profile toward multiple HSP90s chaperones [55]. In such a perspective, more selective approaches are urgently needed. TRAP1 seems to be a central regulatory protein that exerts its balancing functions at the crossroad between different kinds of metabolism during the transformation process and is selectively upregulated by several human malignancies. For these reasons, it can be considered at the same time an attractive target for the development of novel anticancer strategies and a promising study model for the understanding of tumor cells biology at a systemic level.

5 Concluding remarks: from the bench to the bed

HSP90s in general have long been considered targets of interest for the treatment of cancers. However, very few data are available on the selectivity profiles of different HSP90 isoform inhibitors. Several studies have shown that TRAP1 contributes to the overall survival of cancer cells and is up-regulated in most tumour types including, among others, prostate, colorectal, breast, nasopharyngeal cancer. An overview of different in vitro/ in vivo contexts analysed for TRAP1 functions is shown in Table 1. TRAP1 protection against ER stress and apoptosis is functionally linked to both its regulatory activity on mitochondrial transition pore and its role in homeostatic/quality control mechanisms on the ER. Very recently, the crystal structure of full-length TRAP1 has been presented [68], opening new scenarios on the possibility to design novel, specific TRAP1 inhibitors to direct in different subcellular compartments.

However, new paradigm are needed to tackle cancer treatment through a wide biology approach, contemporarily acting on various intersecting pathways. Figure 2 shows the role of TRAP1 in carcinogenesis and its
association/overlap with “classical” hallmarks of cancer (adapted from 69). The potential application of TRAP1 network dissection provides an opportunity to undertake a new strategy to disrupt networks of integrated control in cancer cells. This could lead to the development of new generation molecules, which could both attain to the clinic and help to understand the complexity of tumor biology. In this view, the disruption of the interaction between TRAP1 and its partners could represent a selective strategy to disrupt the whole homeostatic network. Indeed, protein-protein interactions are becoming interesting targets for drug discovery studies because of their importance in signal transduction and in the regulation of cellular functions [70].

Altogether the updated TRAP1 report aims at transferring from bench to bed crucial information for clinicians, molecular biologists involved in cancer research and, more importantly, for patients.

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