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COMPARTMENTALIZED CANCER DRUG DISCOVERY TARGETING MITOCHONDRIAL Hsp90 CHAPERONES

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Abstract

There is a plethora of attractive drug targets in cancer, but their therapeutic exploitation proved more difficult than expected, and only rarely truly successful. One possibility not often considered in drug discovery is that cancer signaling pathways are not randomly arranged in cells, but orchestrated in specialized subcellular compartments. The identification of Heat Shock Protein-90 (Hsp90) chaperones in mitochondria of tumors, but not most normal tissues, provides an example of a compartmentalized network of cell survival, opening fresh prospects for novel, subcellularly-targeted cancer drug discovery.

Keywords

Hsp90; mitochondrial permeability transition; CypD; protein folding; cancer therapy

Cancer drug discovery in the post-Imatinib era

The improved knowledge of cancer genes (Vogelstein and Kinzler, 2004), coupled with the feasibility of disabling specific molecular lesion(s) in tumors (O'Dwyer and Druker, 2000), ushered the era of “targeted” drugs (Sawyers, 2004): molecular “magic bullets” that would change forever the landscape of cancer care. Unfortunately, this expectation met a much harsher reality. The extraordinary complexity of most malignancies with hundreds of mutated or deregulated gene pathways (Wood *et al.*, 2007), the astronomical costs and low success rate (typically 0.0001%) of “target-centric” drug discovery (van der Greef and McBurney, 2005), and the herculean task of fulfilling hundreds of regulatory steps and decision points to bring new agents to the clinic (Steensma, 2009), have greatly hampered the advent of molecular cancer therapeutics. As a result, oncology trial design all too often pursues small, and at times clinically insignificant gains, over 50% of the largest (and costliest) oncology phase III trials fails to produce benefit, and new cancer drug registration has actually fallen by 40% over the past decade (Schein and Scheffler, 2006). These

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CONFLICT OF INTEREST

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disappointments have not gone unnoticed, and patient advocacy groups and media organizations (Kolata, 2009; Leaf, 2004) are urging new venues to improve cancer drug discovery, and produce, if not the “cure”, at least meaningful advances in patient survival.

Pathway-oriented drug discovery and the Hsp90 chaperone network

A fresh approach to these challenges is to take advantage of systems biology tools, and model cancer pathways in their globality (Rajasethupathy *et al.*, 2005). Connectivity maps (Lamb *et al.*, 2006) linking multiple signaling pathways may more accurately recapitulate tumor heterogeneity, uncover redundancy and buffering, and ultimately identify *nodal proteins*, hub molecules integrating multiple downstream mechanisms of cell proliferation, survival, and adaptation (Butcher, 2005; Rajasethupathy *et al.*, 2005). Nodal proteins, for instance the EGF receptor (Citri and Yarden, 2006), offer prime opportunities for cancer drug discovery, as their antagonists may provide genuine *pathway inhibitors*, disabling entire signaling network(s), instead of a single target, and thus suited to overcome tumor heterogeneity (Wood *et al.*, 2007).

The molecular chaperone Hsp90 is another example of a nodal protein (McClellan *et al.*, 2007), intersecting multiple signaling networks (Zhao and Houry, 2007). Hsp90 and Hsp90-like molecules (see below) oversee protein folding quality control (Young *et al.*, 2001), trafficking of signaling molecules at the plasma membrane (Garcia-Cardena *et al.*, 1998), delivery of preproteins to mitochondria (Young *et al.*, 2003), assembly and disassembly of cytoskeletal proteins in the cytosol (Barral *et al.*, 2002), and disassembly of transcriptional complexes in the nucleus (Freeman and Yamamoto, 2002). These functions require sequential cycles of ATPase activity (Prodromou *et al.*, 1997), and recruitment of co-chaperones, including Hsp70, $p50^{cdc37}$, Aha1, $p60^{hop}$, and p23 (Pearl and Prodromou, 2000). Differently from Hsp70, which controls folding of all newly synthesized proteins (Schmid *et al.*, 1994), Hsp90 oversees the maturation and/or stability of client proteins implicated in hormone and growth factor receptor signaling, cell cycle progression, and cell survival (Pearl and Prodromou, 2000; Young *et al.*, 2003). This pathway is exploited in cancer, where Hsp90 chaperoning (Whitesell and Lindquist, 2005), contributes to drug resistance (Cowen and Lindquist, 2005), metastasis (Eustace *et al.*, 2004), tumor cell survival (Rodina *et al.*, 2007), and transcriptional mechanisms of transformation (Dai *et al.*, 2007).

Targeting Hsp90 for novel cancer therapeutics: clinical experience and unanswered questions

In addition to these nodal properties, other features make Hsp90 an almost ideal cancer drug target (Isaacs *et al.*, 2003). Its ATPase pocket can accommodate small molecule inhibitors (Isaacs *et al.*, 2003), and Hsp90 protein levels (Neckers, 2002), as well as ATPase activity (Kamal *et al.*, 2003) are increased in tumors, compared to normal tissues, suggesting that Hsp90 therapeutics may have desirable selectivity (Isaacs *et al.*, 2003). Accordingly, multiple small molecule Hsp90 antagonists have been developed from 17-allylaminogeldanamycin (17-AAG), a less toxic derivative of the benzoquinone ansamycin antibiotic, Geldanamycin (GA) (Isaacs *et al.*, 2003), or, more recently, from purine or

resorcinol structures (Solit and Chiosis, 2008). These agents competitively inhibit the Hsp90 ATPase activity, shut off the chaperone function, and induce degradation of multiple client proteins (Isaacs *et al.*, 2003). In turn, this exerts anticancer activity, predominantly through cell cycle arrest, followed by variable degrees of apoptosis, especially in "sensitive" cell types (Isaacs *et al.*, 2003; Solit and Chiosis, 2008). Despite this promising background, the response to Hsp90 antagonists in the clinic has been in general disappointing (Table 1), certainly inferior to what was expected from potential "pathway inhibitors" (Drysdale *et al.*, 2006). These less-than-impressive clinical data (Table 1) raised questions about drug efficacy, whether Hsp90 is as nodal a target as it was hoped, or, rather, whether other aspects of Hsp90 biology needed to be uncovered in order to unlock the full therapeutic potential of these agents.

Compartmentalization of Hsp90 chaperones in tumor mitochondria

At least one of the missing aspects of Hsp90 biology that may influence the response to therapy is its novel subcellular localization to mitochondria. Originally noted in a global survey of the mitochondrial proteome (Mootha *et al.*, 2003), Hsp90 is abundantly present in mitochondria, and sorted to both the organelle intermembrane space and the matrix (Kang *et al.*, 2007). An Hsp90-like chaperone, Tumor Necrosis Factor Receptor-Associated Protein-1, or TRAP-1 is also a mitochondrial protein (Cechetto and Gupta, 2000; Felts *et al.*, 2000), localized to the organelle matrix (Kang *et al.*, 2007), as well as the intermembrane space (Pridgeon *et al.*, 2007), in vivo. This subcellular localization is selective, in that both Hsp90 chaperones are differentially expressed in mitochondria of tumor cells, while undetectable or expressed at very low levels in the organelles of most normal tissues (see below), in vivo (Kang *et al.*, 2007). The basis for this tumor preference has not been determined, but it may have to do with changes in mitochondrial trafficking in transformed cells. Accordingly, deregulation of cancer signaling pathways, for instance Akt, has been associated with a more avid mitochondrial preprotein import machinery (Wright *et al.*, 2008), and Ras transformation of model fibroblasts, but not glucose deprivation, was sufficient to upregulate Hsp90 levels in mitochondria (Kang *et al.*, 2007).

Once in mitochondria, Hsp90 and TRAP-1 bind the matrix immunophilin, cyclophilin D (CypD) (Kang *et al.*, 2007). These interactions are direct, recapitulated in cell-free systems, and non-overlapping, as TRAP-1-CypD complexes do not contain Hsp90, and vice versa (Kang *et al.*, 2007). CypD is a peptidyl-prolyl *cis, trans* isomerase long recognized as a physical component of a mitochondrial permeability transition pore (Woodfield *et al.*, 1998). Opening of the pore in response to cell death or stress stimuli is thought to mediate an acute increase in organelle conductance to solutes, a process called permeability transition (Green and Kroemer, 2004; Tsujimoto and Shimizu, 2007). In turn, this triggers a cascade of events, including swelling of the matrix, loss of inner membrane potential, remodeling of the cristae, and ultimately rupture of the outer membrane with release of apoptogenic proteins in the cytosol (Green and Kroemer, 2004; Tsujimoto and Shimizu, 2007). There is little doubt that mitochondrial permeability transition initiates cell death. What is less clear is the timing of these steps, how they are interconnected, or even the composition of a permeability transition pore (Halestrap, 2009). For instance, long-held constituents of the pore, such as the voltage-dependent anion channel (VDAC) (Baines *et*

al., 2007), and the adenine nucleotide translocator (ANT) (Kokoszka *et al.*, 2004) turned out to be dispensable for apoptosis, in vivo, and only CypD was required for some, but not all, forms of cell death, in particular those triggered by oxidative stress and Ca^{2+} overload (Baines *et al.*, 2005; Nakagawa *et al.*, 2005).

Hsp90 chaperones as novel regulators of mitochondrial permeability transition

To reconcile these seemingly contradictory views, an alternative model for a *dynamic* permeability transition pore was proposed, in which clusters of misfolded proteins generated in response to oxidative stress acquire the ability to function as a CypD-regulated pore (He and Lemasters, 2002). A prediction of this model was that yet-to-be-discovered molecular chaperones opposed the pore-forming functions of CypD via protein (re)folding mechanisms, and thus preserve mitochondrial integrity (He and Lemasters, 2002). The fact that Hsp90 and TRAP-1 are novel CypD-associated chaperones (Kang *et al.*, 2007) fits well with this scenario. Functional data are also consistent with the model, as acute knockdown of TRAP-1 triggered CypD-dependent apoptosis (Kang *et al.*, 2007; Masuda *et al.*, 2004), whereas overexpression of TRAP-1 protected against oxidative cell death (Hua *et al.*, 2007; Kang *et al.*, 2007; Montesano Gesualdi *et al.*, 2007). As far as pathophysiological relevance, this cytoprotective pathway emerged as an important regulator of neuronal homeostasis. Accordingly, TRAP-1 functions as a survival factor for neurons (Xu *et al.*, 2008), and astrocytes (Volobouéva *et al.*, 2007), via an activating phosphorylation by the PTEN-induced kinase, PINK1 (Pridgeon *et al.*, 2007). In line with this model, PINK1 is also anti-apoptotic in neurons (MacKeigan *et al.*, 2005), countering oxidative damage (Petit *et al.*, 2005), and loss-of-function mutations in this kinase are associated with certain types of familial Parkinson's disease characterized by neuronal wasting (Henchcliffe and Beal, 2008). Whether CypD is the downstream target of TRAP-1 neuroprotection (Pridgeon *et al.*, 2007) is currently not known. What is known, however, is that CypD mediates neuronal cell death after focal cerebral ischemia (Schinzel *et al.*, 2005), and neurodegenerative conditions (Forte *et al.*, 2007), including Alzheimer's disease (Du *et al.*, 2008). As one of only two exceptions in normal tissues, mitochondrial Hsp90 is present in the brain (Kang *et al.*, 2007), but whether it has protective functions for neurons has not yet been tested. Although a PINK1-TRAP-1-CypD axis may regulate the balance of neuronal apoptosis, the converse approach of selectively disabling this pathway in mitochondria may have intriguing implications for cancer therapeutics. Therefore, an obvious question is whether the Hsp90 antagonists currently in the clinic (Table 1) accumulate in mitochondria, and inhibit this compartmentalized pool of the chaperones. Surprisingly, neither GA- (Kang *et al.*, 2007), nor non-GA (Kang *et al.*, 2009)-based antagonists accumulated in tumor mitochondria, suggesting that these subcellular pools of Hsp90 and TRAP-1 escaped inhibition by current Hsp90 therapeutics.

Targeting mitochondrial Hsp90 chaperones for compartmentalized, pathway-oriented cancer drug discovery

The concept of "mitochondrial medicine", aimed at manipulating cell death in human diseases has long been pursued for experimental therapeutics (Armstrong, 2007). Much of the challenge resides in how best to deliver cargos to mitochondria, either to dampen oxidative stress for improved cell survival (Szeto, 2008), or, conversely, trigger permeability transition and cell death (Armstrong, 2007). The latter strategy has appeal for cancer therapeutics because it may *directly* lower a general survival threshold in tumors (Pilkington *et al.*, 2008), regardless of their heterogeneity or genetic makeup (Fig. 1). This approach is a conceptual advance over conventional anticancer regimens, which also activate mitochondrial cell death (Johnstone *et al.*, 2002), but do so *indirectly* as a result of checkpoint activation, DNA damage, etc., and are thus susceptible to compensatory and redundant survival signals (Igney and Krammer, 2002) (Fig. 1). The concept of truly "mitochondriotoxic" agents, *directly* inducing organelle collapse (Fantin and Leder, 2006) (Fig. 1) materialized with the development of small molecule mimetics of pro-apoptotic Bcl-2 proteins (Zeitlin *et al.*, 2008), designed to directly permeabilize the mitochondrial outer membrane, and trigger permeability transition (Green and Kroemer, 2004; Tsujimoto and Shimizu, 2007). These compounds are showing encouraging responses in certain hematologic malignancies (Zeitlin *et al.*, 2008), but the sheer redundancy of Bcl-2 proteins requires simultaneous blockade of multiple members for optimal efficacy (Vogler *et al.*, 2008), and examples of compensatory mechanisms executed by other pro-survival Bcl-2 proteins have been reported (Deng *et al.*, 2007).

To develop a direct mitochondriotoxic strategy targeting Hsp90 chaperones (Fig. 1), proof-of-concept experiments were carried out with Shepherdin, a peptidomimetic inhibitor of the interaction between Hsp90 and one of its client proteins, survivin (Plescia *et al.*, 2005). Shepherdin crosses the plasma membrane via a positively charged *Antennapedia* helix III sequence (Torchilin, 2006) fused at the amino terminus of the peptidomimetic (Plescia *et al.*, 2005). The ability of this moiety to deliver cargos to mitochondria has been debated (Li *et al.*, 2007; Ross *et al.*, 2004), and in any case likely reflects a general property of cross-membrane transfer, rather than a specific mitochondriotropic signal. Through its *Antennapedia* sequence, Shepherdin readily accumulated in all mitochondrial compartments (Kang *et al.*, 2007), and within minutes triggered all the pathophysiological hallmarks of permeability transition, including membrane blebbing, loss of membrane potential, discharge of cytochrome c, and massive cell death (Gyurkocza *et al.*, 2006; Kang *et al.*, 2007). This pathway was mediated by CypD, independently of p53 status or expression of pro-survival Bcl-2 (Kang *et al.*, 2007; Plescia *et al.*, 2005), and correlated with physical binding of Shepherdin to Hsp90 and TRAP-1 inside mitochondria (Kang *et al.*, 2007). In preclinical studies, systemic administration of Shepherdin was feasible, safe (see below), and resulted in inhibition of tumor growth in multiple xenograft models, in vivo (Gyurkocza *et al.*, 2006; Plescia *et al.*, 2005).

Prompted by these results, an approach to deliver a non-peptidyl, small molecule Hsp90 inhibitor selectively to mitochondria was undertaken. This involved combinatorial

chemistry, linking the Hsp90 inhibitor, 17-AAG to lipophilic cations acting as mitochondrial targeting moieties. These were provided either by 1 to 4 tandem repeats of cyclic guanidinium (Fernandez-Carneado *et al.*, 2005), or, alternatively, triphenylphosphonium (TPP) (Armstrong, 2007). The derived combinatorial compounds, designated Gamitrinibs (GA mitochondrial matrix inhibitors), accumulated in mitochondria, abolished Hsp90 ATPase activity (Kang *et al.*, 2009), triggered permeability transition, and caused complete tumor cell killing, in vitro (Kang *et al.*, 2009) (Fig. 1). For concentrations of unconjugated 17-AAG that had no effect in vivo, Gamitrinibs suppressed tumor growth in various xenograft models in mice, with evidence of mitochondrial dysfunction and apoptosis, in situ (Kang *et al.*, 2009). Although Gamitrinibs likely do not discriminate between the two Hsp90 isoforms (α and β), these compounds differ sharply from all other non-subcellularly targeted Hsp90 antagonists for mechanism of action, that is cytotoxic instead of cytostatic (i), direct mitochondriotoxic activity (ii), and lack of effect on Hsp90 outside mitochondria (iii) (Kang *et al.*, 2009). The latter property may reflect a rapid cytosolic transfer of these agents mediated by their mitochondrial-targeting moiety. For instance, suboptimal concentrations of Shepherdin induce a dual phenotype of mitochondrial dysfunction (Kang *et al.*, 2007), as well as inhibition of cytosolic Hsp90 (Plescia *et al.*, 2005), whereas Gamitrinibs are solely mitochondriotoxic, without affecting Hsp90 client protein stability in the cytosol. Because of this subcellular selectivity, Gamitrinibs do not cause compensatory elevation of anti-apoptotic Hsp70 levels (Beere *et al.*, 2000), and are not expected to induce Src phosphorylation (Koga *et al.*, 2006), which may enhance tumor cell invasion and metastasis (Price *et al.*, 2005), two unwanted effects of non-subcellularly targeted Hsp90 inhibitors, i.e. 17-AAG.

Qualitatively different mechanisms of mitochondrial homeostasis in tumor *versus* normal tissues?

A distinctive feature of mitochondria-directed Hsp90 antagonists, Shepherdin (Gyurkocza *et al.*, 2006; Plescia *et al.*, 2005), and Gamitrinibs (Kang *et al.*, 2009), is their safety for normal tissues. Although these agents do accumulate in normal mitochondria (Kang *et al.*, 2009), they have no effect on permeability transition or cell viability, in vitro, and cause no signs of systemic toxicity, alterations in tissue histology, blood chemistry parameters, or bone marrow function, in vivo (Gyurkocza *et al.*, 2006; Kang *et al.*, 2007; Kang *et al.*, 2009; Plescia *et al.*, 2005). Several possibilities may explain this high degree of selectivity, which bodes well for a desirable therapeutic window of these agents. First, mitochondria of most normal tissues do not contain Hsp90 or TRAP-1 (Kang *et al.*, 2007), and this may sharply reduce their sensitivity to Gamitrinib or Shepherdin (Fig. 2). Similar to their cytosolic counterparts (Kamal *et al.*, 2003), it is also possible that tumor-associated mitochondrial Hsp90s exhibit a 100-fold increase in ATPase activity compared to normal tissues, further broadening the selectivity of these antagonists. Different kinetics of mitochondrial accumulation may also play a role, and the higher mitochondrial membrane potential of tumor cells compared to normal tissues (Chen, 1988), may facilitate a 10-fold preferential accumulation of Gamitrinibs, especially Gamitrinib-TPP, in mitochondria of tumor *versus* normal cells (Murphy, 2008). And, finally, there is the possibility that additional regulatory molecules, perhaps co-chaperones, be similarly recruited to tumor mitochondria and

cooperate with Hsp90 protein (re)folding to oppose CypD (Fig. 2). The identity of these putative accessory molecules is unknown, but this model may explain why Gamitrinibs are apparently safe even for normal tissues that do contain mitochondrial Hsp90, for instance the brain (Kang *et al.*, 2007). In this context, it would be the lack of other tumor-associated regulators of the mitochondrial Hsp90 network that reduce the sensitivity of these tissue(s) to the compounds.

Regardless of the mechanism, or mechanisms underlying tissue selectivity, one general hypothesis emerges from these findings, that the machinery controlling CypD pore-forming properties may be qualitatively different in tumor *versus* normal mitochondria (Fig. 2). In this context, it is possible that tumor cells commandeer a protective network of mitochondrial Hsp90 chaperones operative in selected normal tissues, where CypD function must be tightly controlled, for instance the brain (Forte *et al.*, 2007; Schinzel *et al.*, 2005). For tumor cells, the survival advantage conferred by this pathway would be almost ideal, specifically preventing CypD-mediated apoptosis triggered by oxidative stress, a condition invariably associated with tumor growth, in vivo (Fig. 2). This model may also explain a second key feature of mitochondria-directed Hsp90 inhibitors, namely their broad efficacy in so many unrelated tumor cell types. This suggests that transformed cells may become dependent, or "addicted" (Weinstein and Joe, 2006) to a steady-state, anti-oxidant survival threshold maintained by mitochondrial Hsp90 chaperones. Following this logic, and as observed experimentally, acute loss of this protective mechanism by mitochondria-targeted Hsp90 inhibitors cannot be compensated for, resulting in sudden organelle collapse and cell death, regardless of the genetic makeup of the tumor (Fig. 2).

Concluding remarks -mitochondrial medicine revisited

Taken together, these recent observations open intriguing opportunities. From a mechanistic perspective, there has been a tremendous effort to map the regulators of mitochondrial permeability transition (Green and Kroemer, 2004; Tsujimoto and Shimizu, 2007), and the role of some of the key players, for instance pro-apoptotic Bcl-2 family proteins, is now firmly established. Are now chaperones, co-chaperones and overall protein (re)folding mechanisms new chapters in the evolving saga of mitochondrial homeostasis (He and Lemasters, 2002), especially when it comes to oxidative stress? And if this is, in fact, a *cancer signaling network*, rather than the activity of individual molecules, which other players or co-chaperones cooperate with Hsp90s in taming CypD-mediated pore formation in tumor mitochondria? From a disease-relevant perspective, it is encouraging to see that a long pursuit of "mitochondrial medicine" (Armstrong, 2007), is finally paying off with attractive drugs in the clinic (Zeitlin *et al.*, 2008), and other new agents, i.e. Shepherdin and Gamitrinibs (Gyurkocza *et al.*, 2006; Kang *et al.*, 2009; Plescia *et al.*, 2005) on the horizon. Because of the flexible combinatorial platform of Gamitrinibs, a similar approach could be envisioned to direct smaller, purine- or resorcinol-based Hsp90 antagonists (Solit and Chiosis, 2008) to mitochondria, thus potentially further improving on their anticancer activity. In addition, the concept of directing therapeutic agents to specialized subcellular compartments need not be exclusively limited to mitochondria. As shown by the proof-of-concept data with Gamitrinibs (Kang *et al.*, 2009), compartmentalized inhibition of signaling pathways could dramatically expand the repertoire of cancer drug discovery as a

whole. This may mean fewer costly and low-yield screening efforts, generation of agents with new specificity and mechanism of action, and concrete therapeutic prospects for targeting cancer signaling pathways that are currently considered not “drugable”.

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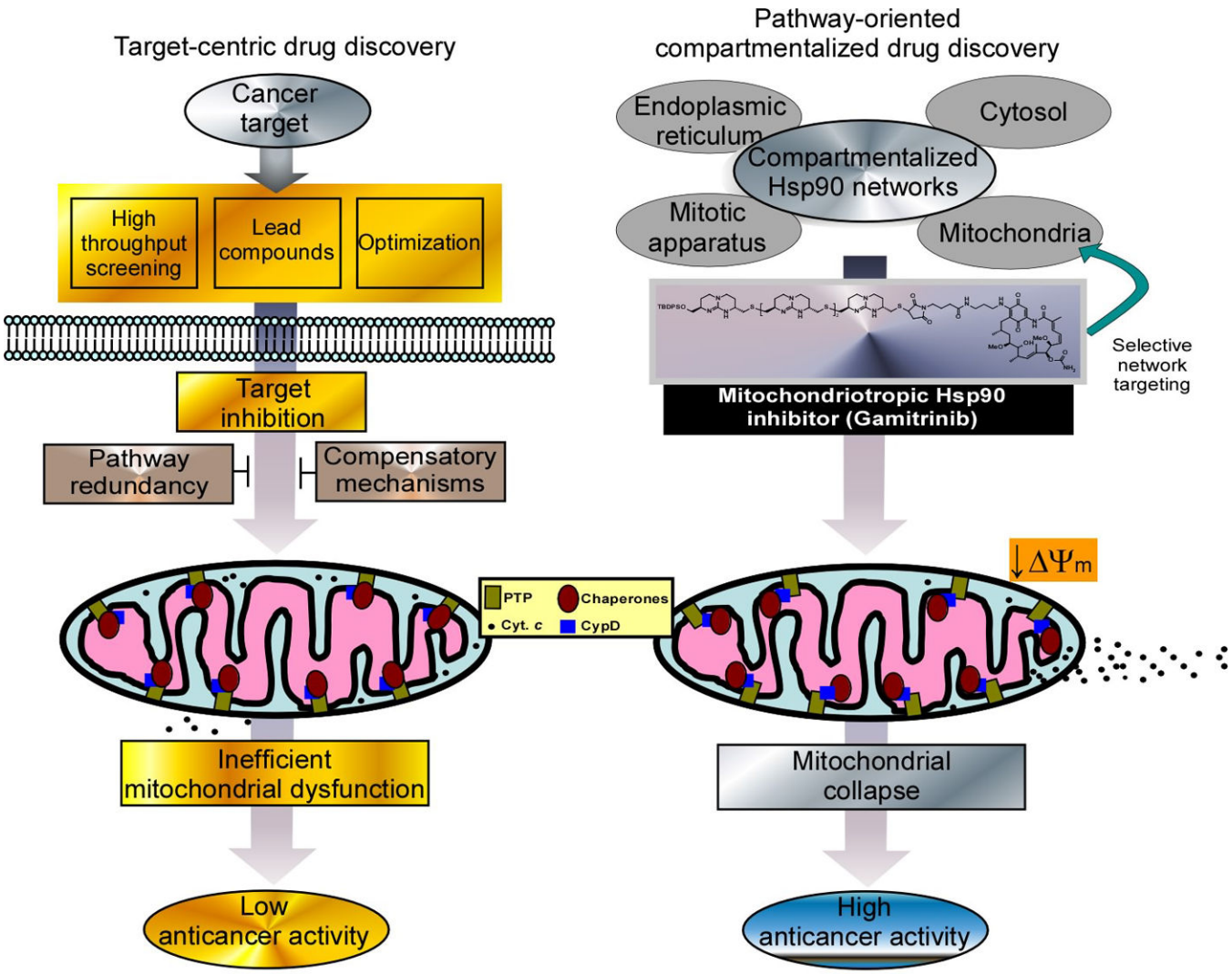

Fig. 1. Mitochondriotoxic agents for cancer therapy

'Target-centric' cancer drug discovery (*left panel*) involves the selection of a target gene, and the generation of inhibitors by high throughput screening, and lead optimization. These agents may inhibit the target, and cause *indirect* activation of mitochondrial cell death, in vivo, but anticancer activity is often reduced by pathway redundancy and compensatory prosurvival signals. Conversely, combinatorial engineering of pathway inhibitors, for instance 17-AAG, to target a subcellular cancer network, i.e. mitochondria (*right panel*), generates antagonists that *directly* induce organelle collapse, and enhanced anticancer activity, bypassing compensatory survival mechanisms. ΔΨm, mitochondrial membrane potential, PTP, permeability transition pore; cyt c, cytochrome c, CypD, cyclophilin D.

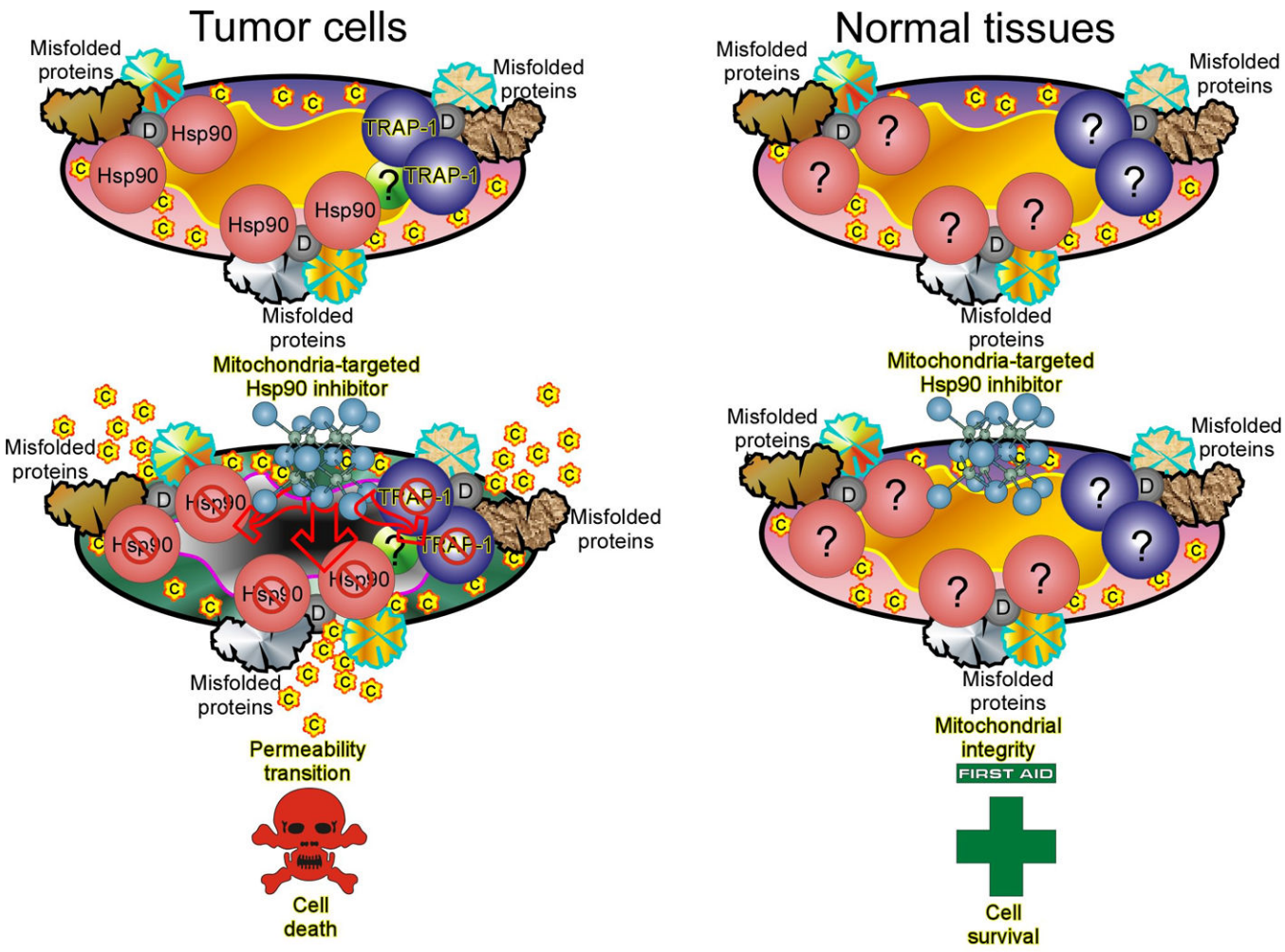

Fig. 2. Qualitative differences in mitochondrial homeostasis in normal and tumor cells

Mitochondria of tumor cells (*left panel*) exploit an Hsp90 chaperone protein refolding network to suppress Cyclophilin D (CypD)-mediated permeability transition. This may involve CypD participation in an organized permeability pore, or as a regulator of clustered, misfolded proteins formed in response to oxidative damage that acquire pore conductance (shown in the figure). Inhibition of organelle Hsp90 ATPase activity by mitochondria-directed Hsp90 antagonists (Shepherdin, Gamitrinibs) results in permeability pore opening, and CypD-dependent cell death. Conversely, mitochondria of most normal cells (*right panel*) are devoid of Hsp90 chaperones, and potentially also of regulators/co-chaperones in this pathway, suggesting that alternative mechanisms control CypD pore functions. This makes normal tissues insensitive to the action of mitochondria-targeted Hsp90 antagonists. C, cytochrome c; D, CypD.

Table 1

Clinical experience with current small molecule Hsp90 antagonists.

Drug	Trial	Patients	Regimen	Outcome	Citation
17-AAG	Phase I	21	Single agent	NR	J Clin Oncol 23:1078
17-AAG	Phase I	19	Single agent	3 SD	J Clin Oncol 23:1885
17-AAG	Phase I	30	Single agent	2 SD	J Clin Oncol 23:4152
17-AAG	Phase I	45	Single agent	NR	Clin Cancer Res 11:3385
17-AAG	Phase I	13	Single agent	NR	Clin Cancer Res 12:6087
17-AAG	Phase II (RCC)	20	Single agent	NR	Invest. New Drugs 24:543
17-AAG	Phase I	54	Single agent	NR	Clin Cancer Res 13:1775
17-AAG	Phase I	44	Single agent	NR	Clin Cancer Res 13:1769
17-AAG	Phase I	15 (Ped)	Single agent	NR	Clin Cancer Res 13:1783
17-AAG	Phase I	17 (Ped)	Single agent	NR, 5 SD	Clin Cancer Res 13:1789
17-AAG	Phase I	25	plus Trastuzumab	1 PR, 4 MR 4 SD	J Clin Oncol 25:5410
17-AAG	Phase I	25	plus Paclitaxel	NR, 6 SD	Clin Cancer Res 14:3456
17-AAG	Phase I	27	plus Irinotecan	NR, 11 SD	Clin Cancer Res 14:6704
17-AAG	Phase II (HRPC)	15	Single agent	Trial discontinued	Clin Cancer Res 14:7940
17-AAG	Phase II (MM)	15	Single agent	NR	Clin Cancer Res 14:8302
IPI-504	Phase III (GIST)		Single agent	Trial discontinued	http://investor.ipi.com/releasedetail.cfm?ReleaseID=377328
IPI-504	Phase II (HRPC)		Single agent	Trial discontinued	http://biz.yahoo.com/pz/080714/146331.html

Ped, pediatric patients; RCC, renal cell carcinoma; HRPC, hormone-refractory prostate cancer; MM, malignant melanoma; GIST, gastrointestinal stromal tumor; NR, no objective response; PR, partial response; MR, minor response; SD, stable disease.