



Review

Hsp90 inhibitors and drug resistance in cancer: The potential benefits of combination therapies of Hsp90 inhibitors and other anti-cancer drugs

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ABSTRACT

Hsp90 is a chaperone protein that interacts with client proteins that are known to be in the cell cycle, signaling and chromatin-remodeling pathways. Hsp90 inhibitors act additively or synergistically with many other drugs in the treatment of both solid tumors and leukemias in murine tumor models and humans. Hsp90 inhibitors potentiate the actions of anti-cancer drugs that target Hsp90 client proteins, including trastuzumab (HerceptinTM) which targets Her2/Erb2B, as Hsp90 inhibition elicits the drug effects in cancer cell lines that are otherwise resistant to the drug. A phase II study of the Hsp90 inhibitor 17-AAG and trastuzumab showed that this combination therapy has anticancer activity in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. In this review, we discuss the results of Hsp90 inhibitors in combination with trastuzumab and other cancer drugs. We also discuss recent results from yeast focused on the genetics of drug resistance when Hsp90 is inhibited and the implications that this might have in understanding the effects of genetic variation in treating cancer in humans.

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1. Introduction

Heat shock protein 90 Hsp90, a protein of molecular weight 90 kDa that is conserved from yeast to humans, is a molecular

chaperone with over 200 identified client proteins. Hsp90 is an especially promising target for anti-cancer drugs as many of its client proteins are present in pathways that are often disrupted in many types of cancers [1]. A list of Hsp90 client proteins can be found at (<http://www.picard.ch/downloads/Hsp90facts.pdf>). Client proteins include apoptotic factors, protein kinases, transcription factors, and signaling proteins. Some client proteins, like steroid receptors [2–6], epidermal growth factor receptor (EGFR) family members [7], the MET oncogene [8,9], Raf-1 kinases [10],

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AKT kinases [11], BCR-ABL fusion proteins in leukemia [8,12–14], mutant p53 [15], cyclin dependent kinase 4 (CDK4) [16–18], hypoxia-inducible factor 1 α (HIF1 α), matrix metalloproteinase 2 (MMP2) [19], and chromatin-remodeling proteins such as the histone deacetylases (HDACs) [20–24] and SMYD3 [25,26] are often mutated in cancer cells.

While Hsp90 is required in all cells, tumor cells are especially sensitive to Hsp90 inhibitors as they are “oncogene addicted” and require especially high levels of Hsp90 [27–31]. Genetically unstable cancer cells live under a multitude of stresses, including mutated and amplified signaling and client proteins, chromosome and microsatellite instability and aneuploidy, hypoxia, low pH, and low nutrient concentrations [32–36]. Cancer cells can survive and thrive in stressed microenvironments by quickly selecting for adaptive mutations and chromosomal rearrangements that increase their survival and proliferative abilities.

Unfortunately, the effectiveness of anti-cancer drugs that specifically target individual cancer promoting proteins or signaling pathways may be gradually decreased, or even totally lost, due to the genetic and epigenetic variation in cancer cells, as they become drug resistant. One strategy to address this problem is to identify targets, such as Hsp90, the proteasome [37], and the autophagosome [38–40], that affect multiple signaling pathways or the basic machinery required for cancer cells to survive under stress.

Inhibition of Hsp90 functions affects multiple oncogenic substrates simultaneously and has shown obvious anti-cancer effects in vitro and in vivo. One Hsp90 inhibitor, 17-allylamino, 17-demethoxygeldanamycin (17-AAG), a geldanamycin analog, has completed phase II clinical trials in a number of cancers [41–47] (see <http://www.clinicaltrials.gov> for a list of many clinical trials). Geldanamycin and its derivatives, as well as structurally different compounds like radicicol [48], are N-terminal Hsp90 inhibitors that interfere with the ATP-binding domain of Hsp90. Many C-terminal Hsp90 inhibitors are under preclinical development including several novobiocin-based [49] and coumarin-based inhibitors [50].

Chemotherapy and radiation therapy [51] remain the most commonly used treatments for cancer, but new and more specific anti-cancer drugs are emerging. However, due to the rapid genetic and epigenetic changes in adaptation to stress induced by anti-cancer drugs, cancer cells are often able to become resistant to single or multiple anti-cancer agents [52–54]. The development of resistance is especially serious with chemotherapy and radiation therapy, and a critical goal of cancer therapy is to more effectively combat this resistance. Drug resistance can be induced by decreasing the uptake of water-soluble drugs, changing the activity of cytotoxic drugs by covalent modifications, by oxidation [55–58], glutathionylation [59], and glucuronidation [60], and by increasing the efflux of hydrophobic drugs [52–54].

2. Hsp90 and the evolution of new phenotypes

Hsp90 aids in the folding of many signaling proteins under basal conditions, and in environmental stress, such as in cancer cells. In all eukaryotes studied, from fungi to mammals, Hsp90 and its orthologs are among the most abundant proteins comprising 1–2% of the total proteins under normal conditions [61]. Hsp90 is unique among the protein chaperones as its client proteins are primarily signaling molecules, such as nuclear-hormone receptors, tyrosine kinases, and chromatin-remodeling proteins [62–64]. It is termed a “heat shock protein”, but actually Hsp90 has high constitutive protein levels that are induced approximately 2-fold during environmental stress [65–68]. For example, yeast, which is presumably similar to human cells in this respect, has 445,000 molecules of Hsp82 (stress-induced Hsp90) per cell and upon stress this amount may be increased by 2-fold. This is in comparison to many kinases and transcription factors in both

yeast and human cells which have fewer than 10,000 molecules per cell (<http://yeastgfp.yeastgenome.org/>). During stress, Hsp90 protein levels are higher, but its chaperone activity is functionally titrated by the increase in the level of unfolded signaling proteins, co-chaperones, and post-translational modifications [69].

Hsp90 has been postulated to have a major role in facilitating the rapid evolution of new traits. In *Drosophila* and *Arabidopsis*, it is viewed as a “capacitor” for morphological evolution because reducing Hsp90 levels during early development produces a multitude of new phenotypes by unmasking hidden phenotypic variation in adults [69–72]. It has been proposed that the variation is unmasked because numerous signaling molecules that are involved in morphological development are targets of Hsp90 and, consequently, have altered activity when Hsp90 levels are reduced [71,72]. Several generations of selection of the unmasked new phenotype enriches the polymorphisms that contribute to the phenotype by genetic rearrangement, ultimately leading to a stable phenotype even in the absence of stress [71,72].

Our laboratory has shown in *Drosophila* that reduction of Hsp90 activity can epigenetically unmask new phenotypes, even in the absence of genetic variation [73]. We thus propose that epigenetic induction of new phenotypes by stress can facilitate the genetic rearrangement required to permanently stabilize the new phenotype in the selected population [74–77]. We also propose that epigenetic induction of new phenotypes by stress is mutagenic and that this can allow the stochastic induction of new mutations that can stabilize the new phenotype in the selected population [74–77]. Recently, Gangaraju and colleagues showed that Hsp90 reduction epigenetically activates transposons in *Drosophila* by inactivation of the Piwi protein, an Argonaute-family protein that is involved in the microRNA pathway of RNA-directed chromatin repression [78]. In other words, Hsp90 can facilitate evolution of the organism, as well as the cancer cell, by both epigenetic and genomic mechanisms.

In 2005, Cowen and Lindquist showed that high levels of Hsp90 facilitated the evolution of drug resistance in diverse species of fungi by altering the activities of mutated drug resistance genes [70]. We also proposed that Hsp90 might have a similar effect in the development of drug resistance in cancer cells [79,80].

3. Synergistic effects of hsp90 inhibitors and other anti-cancer drugs

Recent preclinical and clinical studies explored the effects of a combination of Hsp90 inhibitors and other anti-cancer agents in cancer therapy. Based on the different therapeutic mechanisms of conventional anti-cancer drugs, Hsp90 inhibitors exerted different effects in these combinational studies. Additive or synergistic effects were observed in most cases (Table 1).

Preclinical data from different cancer cell lines and tumor xenograft models indicate that Hsp90 inhibitors show additive or synergistic effects in killing cancer cells when combined with most conventional cytotoxic agents (such as taxanes, cisplatin, gemcitabine and cytarabine), proteasome inhibitors, HDAC inhibitors, and new molecular targeting agents in schedule-and-cell-type-dependent manners (Table 1).

Combination therapies of Hsp90 inhibitors and other drugs are now in phase II clinical trials. A recently completed phase II study of 17-AAG, an Hsp90 inhibitor, and trastuzumab, showed that this combination therapy has significant anticancer activity in patients with HER2-positive metastatic breast cancer progressing on trastuzumab [42]. In this study, 31 breast cancer patients progressing on trastuzumab were enrolled with a median age of 53 years and a minimum Karnofsky performance status (KPS) of 90% [42]. The KPS attempts to quantify cancer patients' general well-being and activities of daily life and is used in oncological

Table 1

Additive/synergetic effects of Hsp90 inhibitors and other anti-cancer drugs.

Hsp90 inhibitors	Drugs in combination	Interaction	Cancer cell type	Mechanism; clinical trial (if performed)	References
GA/17-AAG	Cisplatin	Synergetic	Colon (in HCT116 cell); antagonize in HT29 cell; solid tumors	Schedule-dependent; p53, JNK pathway involved; phase 2 for solid tumors	[91,92,174]
Radicalcol	Cisplatin, oxaliplatin	Synergistic	Colon; glioma	MLH1 proficient/deficient; no change in apoptosis/cell cycle	[94,175]
GA	Cisplatin	Synergistic	Pediatric solid tumor (neuroblastoma, osteosarcoma); hepatoma;	Depletion of Akt, IGF1R; cell cycle arrest;	[93,95]
17-AAG	Taxol	Synergistic (schedule dependent: together)	Breast cancer (mice Xenograft tumors (BT-474) and cell culture)	Down-regulate Akt and Her2	[82,85]
17-AAG	TRAIL receptor antibody (HHGS-ETR1/2)	Synergistic	Hodgkin's lymphoma, colon, prostate	Down-regulate Akt, Erk, cell cycle arrest and death; suppression of NF- κ B pathway (RIP and IKK degradation)	[101–104,176]
17-AAG	Bortezomib (PS-341, proteasome inhibitor)	Synergistic	Breast (MCF-7 cell), leukemia, multiple myeloma	Administration: simultaneously better than sequential addition; accumulation of aggregates	[41,97,98,177]
17-AAG	UCN-01 (7-hydroxystaurosporine), chk1 inhibitor	Synergistic	Leukemia (cell culture, U937, etc.)	Interruption of RAF/MEK and Akt pathways	[117]
17-AAG	Imatinib mesylate/PD180970	Enhancement	Leukemia (CML)	Decrease Bcr-Abl	[114,177–179]
17-AAG	PKC412 (FLT3 tyrosine kinase inhibitor)	Synergistic	AML (cell culture)	Down regulate FLT3, Akt, Erk, STAT5; selective for AML with mutated FLT3	[113]
17-AAG	GTP14564 (FLT3 tyrosine kinase inhibitor against)	Synergistic	Leukemia (leukemias with FLT3 mutations)	Reduced level of FLT3, STAT5, enhanced G0/G1 arrest and apoptosis in leukemia with FLT mutations	[180]
17-AAG	LY294002 (PI3K inhibitor)	Synergistic	Malignant glioma	Down regulate PI3K/Akt	[116,177,181]
17-AAG	Gemcitabine (only effective in S phase cells)	Sensitize	Ovarian tumor, myeloid leukemia cell line	17-AAG arrests cells in G ₁ and G ₂ ; deplete chk1; phase 1 for solid tumors	[174,182]
GA, radicalcol	Topoisomerase II poison (etoposide) (VP16)	Synergistic	Colon (HCT 116)	DNA damage; topoisomerase II activity increase	[120,183]
17-AAG	LBH589 (HDACI)	Synergistic	CML, AML	Attenuate levels of mutant Bcr-Abl	[111]
17-AAG	Histone deacetylase inhibitors (HDACIs): suberoylanilide hydroxamic acid (SAHA) and sodium butyrate (SB)	Synergistic	Leukemia cell lines: human U937, human promyelocytic (HL-60) and lymphoblastic (Jurkat) leukemia cells	Multiple perturbations in signaling, cell cycle, and survival pathways that culminate in mitochondrial injury and apoptosis. Through ERK activation and p21 ^{CIP1} , not Akt	[21,110,184]
17-AAG	ATO (arsenic trioxide)	Synergistic	Leukemia	Abrogate Akt activation, increased ROS generation	[118,177]
17-AAG	Cytarabine	Synergistic	Leukemia (AML)	Chk1 depletion	[119,185]
17-AAG	Doxorubicin	Synergistic	Breast cancer (cell culture)	Not RB and schedule dependent	[85,186]
17-AAG	Trastuzumab (Herceptin), first humanized antibody, target ErbB2	Enhancement at lower doses in TR sensitive cells; no enhancement in TR resistant cells (same as 17-AAG)	Breast	Reduce ErbB2 levels; phase 2 clinical trial for breast cancer	[42,115,187]
Radicalcol	Emunin, NZ28 (inhibit heat shock response)	Sensitize	MM.1s myeloma	Reduced Hsp70 level	[121]

randomized controlled trials as a measure of quality of life. The KPS runs from 0% (dead) to 100% (healthy with no problems).

The exciting results with 17-AAG and trastuzumab in treating trastuzumab-resistant breast cancer, combined with the other Hsp90 combination preclinical trials in rodents, suggests that many more clinical trials will be attempted in the near future.

3.1. Taxanes

Paclitaxel (Taxol[®]) is a mitotic inhibitor used in cancer chemotherapy. It stabilizes microtubules, thereby causing mitotic arrest and apoptosis [81]. Taxol is one of the two clinically available taxanes and is used against a broad range of cancers. Hsp90 inhibitors, such as 17-AAG and geldanamycin (GA), sensitize lung and breast cancer cells to paclitaxel induced cytotoxicity both in vitro and in vivo [82–86]. Low doses of 17-AAG enhance paclitaxel cytotoxicity by drastic reduction of paclitaxel 50% inhibitory concentration (IC₅₀) values and significantly increase induction of apoptosis.

The synergistic effects of 17-AAG and other drugs are dependent on the cell type [82,84,85]. In cells expressing retinoblastoma (RB), or high level of ErbB2 or Akt, that are clients of Hsp90, concurrent exposure to 17-AAG and paclitaxel is required for the synergistic activity of the two drugs. Exposure of these cells to 17-AAG causes a G1 growth arrest [82,85,87], whereas paclitaxel arrests the cells in mitosis. Thus, in future development of combinational treatment strategy, the administration schedule should be considered if cell cycle dependent changes are involved in modulating the activity of the drug.

3.2. Cisplatin

The compound cis-PtCl₂(NH₃)₂ (cisplatin), also known as Peyrone's salt [88], is used to treat several types of cancers, including sarcomas, carcinomas, lymphomas, and germ cell tumors. Cisplatin crosslinks DNA and consequently trigger apoptosis [89,90]. It has been widely used alone or in combined regimes with other anti-cancer drugs for the therapy of a variety of tumors and often shows synergistic anti-cancer effects in different cancer types [91–95]. Of the 17-AAG and cisplatin combinations, synergistic anti-cancer activities were observed in several colon cancer cell lines [91,92], pediatric solid tumor cells cultures (neuroblastoma and osteosarcoma) [95], and hepatoma cell cultures and xenograft models [93].

Radical, another widely used Hsp90 inhibitor, also sensitizes colon cancer cells to cisplatin via the interaction of Hsp90 with MLH1, a protein crucial for DNA mismatch repair [94]. It has been proposed that synergistic interactions depend on the effect exerted by 17-AAG on cisplatin-induced signaling through the JNK stress-induced and the p53 DNA-damage-induced pathways [91,92]. Cisplatin and Hsp90 inhibitors like 17-AAG, might be important in inducing cytoprotective effects, thereby lowering the toxicity of chemotherapeutic agents such as gemcitabine [96].

3.3. Proteasome inhibitors

Bortezomib (PS-341; Velcade[™]) is the first proteasome inhibitor approved for the treatment of relapsed multiple myeloma (MM) and mantle cell lymphoma (MCL). In MM, complete responses have been obtained in patients with otherwise rapidly advancing disease [41,97,98]. The attributing mechanisms include increased protein misfolding, coupled to impaired protein clearance by suppression of the chymotryptic activity of the 20S proteasome. The marked anti-cancer activity of a combination of Hsp90-and-proteasome inhibitors might arise from their complementing abilities to simultaneously trigger intracellular accu-

mulation of unfolded proteins and preventing their cellular protection functions [41]. More importantly, combined Hsp90-and-proteasome-inhibitors treatment overcomes the drug resistance of primary MM cells which are resistant to cytotoxic chemotherapy and bortezomib [41].

3.4. Death receptor ligands: tumor necrosis factor (TNF) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

TRAIL binds to the death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2) and induces caspase-8-dependent apoptosis. It also binds the receptors DcR1, a decoy receptor and DcR2, which contains a truncated death domain and activates NFκB. The apoptosis-inducing “death receptor” ligands, TRAIL and TNF, are promising candidates for cancer treatment but display variable cytotoxicity and drug resistance in different cell lines [99]. Combination of 17-AAG with “death receptor” targeting agents can synergistically increase their anti-tumor activities and abolishes the drug resistance of TRAIL/TNF in glioma [100]. In TRAIL/TNF-resistant cancer cell lines, such as prostate LNCaP cells, and colon HT29 and RKO cells, pre- or co-exposure to 17-AAG with TRAIL/TNF induced high levels of apoptosis. This was also observed with TNF-resistant lung H23 and H460 cells [46–48]. In all instances, synergistic induction of apoptosis by pre- or co-exposure to 17-AAG with TRAIL/TNF was induced primarily through down regulation of NFκB or Akt cell survival pathways [101–103]. Synergistic effects between 17-AAG and anti-TRAIL monoclonal antibodies have also been observed [104].

3.5. Histone deacetylase inhibitors

HDAC inhibitors, or more accurately “protein deacetylase inhibitors” because they often target proteins other than histones, are a group of compounds that inhibit the deacetylation of many proteins, including histones and Hsp90 [105,106]. HDAC inhibitors can induce apoptosis in cancer cell lines and some HDAC inhibitors are under clinical evaluation [107–109]. Co-administration of 17-AAG with HDAC inhibitors, like sodium butyrate (SB), suberoylanilide hydroxamic acid (SAHA), or LBH589, can synergistically induce apoptosis in leukemia cells [110,111]. Moreover, a combination treatment of 17-AAG and LBH589 is effective in imatinib mesylate (IM)-resistant primary chronic myeloid leukemia blast crisis (CML-BC) and acute myeloid leukemia (AML) cells [111]. The detailed mechanisms of these synergistic effects are unclear, but they likely involve perturbations of survival pathways and cell cycle progression. HDAC inhibitors also leads to Hsp90 hyper-acetylation that inhibits its ATP-binding and chaperoning activities [105].

3.6. Protein kinase inhibitors

Several protein kinase inhibitors (PKIs) act synergistically with Hsp90 inhibitors in killing tumor cells. Leukemic cells with FLT3 tyrosine kinase gain-of-function mutations are synergistically and selectively sensitive to 17-AAG and FLT3 tyrosine kinase inhibitors, midostaurin (PKC412) and GTP14564 [112,113]. Imatinib, a BCR-ABL tyrosine kinase inhibitor, also shows synergistic effects with 17-AAG in imatinib-resistant CML cells over-expressing BCR-ABL and P-glycoprotein [114]. 17-AAG combined with trastuzumab, the humanized antibody against receptor tyrosine kinase ErbB2, inhibits proliferation of trastuzumab-resistant breast tumor cell line JIMT-1 [115].

The molecular mechanisms of these synergistic effects are the pronounced reduction in protein level and activity of these kinases, which are all Hsp90 “client” proteins. Additional synergistic interactions occur when 17-AAG is combined with Chk1 inhibitor UCN-01 or PI3K inhibitor LY294002, and interference with the Akt

survival pathway and cell cycle progression are thought to contribute to the phenomenon [116,117].

3.7. Other drugs and treatments

Hsp90 inhibitors also synergistically act with many other anti-cancer drugs, including doxorubicin, topoisomerase II inhibitors, cytarabine, arsenic trioxide and compounds that inhibit the induction of heat shock proteins, via different mechanisms [86,118–121]. Of the other anti-cancer treatments, such as ionizing irradiation, adding Hsp90 inhibitors also enhances the cancer-killing effects synergistically [122–124]. Generally, treatment with 17-AAG provides a means of reversing the drug or radiation resistance in cancer cells.

4. Natural variation effects Hsp90-dependent drug resistance and sensitivity

Hsp90 ... provide[s] at least two routes to the rapid evolution of new traits: (i) Acting as a potentiator, Hsp90s folding reservoir allows individual genetic variation to immediately create new phenotypes; when the reservoir is compromised, the traits previously created by the potentiated variants disappear. (ii) Acting as a capacitor, Hsp90s excess chaperone activity buffers the effects of other variants, storing them in a phenotypically silent form; when the Hsp90 reservoir is compromised, the effects of these variants are released, allowing them to create new traits. - Jarosz and Lindquist (2010) [69]

In 1958, Schabel suggested that model organisms such as yeast and bacteria can be used to understand drug resistance in cancer [125]. For the past two decades, the Lindquist laboratory [69], the Picard laboratory [126–128], and other laboratories (e.g., [65,129,130]), based on Schabel's advice have used the yeast *Saccharomyces cerevisiae* to understand how Hsp90 affects resistance or sensitivity. In a previous review [80], we discussed how the Lindquist laboratory's Hsp90-based drug-resistance studies might apply to drug resistance in cancer.

Natural variation *S. cerevisiae* can affect the growth rate of the yeast cells [69]. Jarosz and Lindquist have reported that Hsp90 can act either as a "potentiator" or a "capacitor" for drug resistance and considered how this might affect the rapid evolution of new traits in general. Using recombinant inbred lines of bakers' yeast (BY4716) and red wine yeast (RM11-1a) in the presence of anti-fungals, osmotic stressors, and other small molecules, they compared the growth rates in the presence or the absence of Hsp90 [69]. Hsp90 was inhibited by the Hsp90 inhibitors radicicol and geldanamycin [69]. Mechanistic models for the Hsp90-mediated potentiation or capacitation that may explain three of the findings described by Jarosz and Lindquist, rapamycin, hydroxyurea, and 1-chloro-2,4-nitrobenzine (CDNB), are shown in Fig. 1.

The immunosuppressant rapamycin can prolong the life of mice [131–133] and *Drosophila* [134] and is also useful for treating breast and skin cancers [135–138]. Jarosz and Lindquist found that BY4716 and RM11-1a yeast, and all recombinant inbred lines made from these two strains, have identical growth rates in the presence of Hsp90, but RM11-1a yeast have a ~3-fold increase in growth rate in the absence of Hsp90 compared with BY4716 [69]. The recombinant inbred lines made from BY4716 and RM11-1a indicates that the *NFS1* gene must have the RM11-1a genotype to confer rapamycin resistance (Fig. 1a). Nfs1 protein is a cysteine desulfurase that acts as a sulfur donor in tRNA thiolation [139], and yeast mutations in this same pathway confer rapamycin resistance [140].

Jarosz and Lindquist [69] have proposed that the Nfs1 protein is a client for Hsp90 and that Hsp90 folds the Nfs1 into a form that makes both RM11-1a and BY4716 yeast sensitive to rapamycin

(Fig. 1a, left). However, in the absence of Hsp90, Nfs1 with the RM11-1a genotype folds into a new conformation that is now resistant to rapamycin, but the BY4716 genotype protein remains in the rapamycin-sensitive conformation (Fig. 1a, right). In other words, Hsp90 functions as a capacitor for the rapamycin resistant phenotype in the RM11-1a strain but not the BY4716 strain. In the absence of Hsp90, such as during stress, the previously hidden phenotype of rapamycin resistance is revealed by the new of the Nfs1-resistant (*NFS1^R*) conformation in the RM11-1a strain (Fig. 1a).

Hydroxyurea is used to treat a variety of cancers, from leukemia to breast cancer [141–144]. It is also used in combination with other drugs to treat head and neck cancer [145]. One mechanism of action is thought to be through the inhibition of deoxyribonucleotide synthesis [146,147]. Jarosz and Lindquist found that RM11-1a yeast are more resistant to hydroxyurea than BY4716 yeast in the presence of Hsp90, but that both BY4716 and RM11-1a yeast are resistant to hydroxyurea in the absence of Hsp90 [69]. Analyses of the RM11-1a and BY4716 recombinant inbred lines indicate that the *MEC1* gene from BY4716 confers the sensitivity to hydroxyurea (Fig. 1b, left). Mec1 is a component of several checkpoint and DNA repair pathways in yeast [148–151], and therefore likely repairs the DNA damage induced by hydroxyurea.

Jarosz and Lindquist [69] further propose that Hsp90 functions as a capacitor in BY4716 yeast to make the Mec1 protein sensitive to hydroxyurea. However, according to their model, Hsp90 is not a chaperone for the Mec1 protein from RM11-1a yeast, but is a chaperone for Mec1 protein in BY4716 yeast (Fig. 1b). In the absence of Hsp90, such as in stressful environments, the Mec1 protein in BY4716 yeast folds into a different conformation that is now more resistant to hydroxyurea (Fig. 1b, right). Since the Mec1 protein in RM11-1a yeast is not a client for Hsp90, according to their model, it confers resistance to hydroxyurea regardless of whether Hsp90 is present or not (Fig. 1b, right). This result is important because it suggests that what might be a client protein for Hsp90 in one genetic background might not be a client in another genetic background. If this is true in humans, which is likely, this would suggest a possible reason why Hsp90 inhibitors are more effective in some cancer patients than others when used in combination with other drugs (Fig. 1b).

CDNB, a.k.a., DNCB (2,4-dinitro-1-chlorobenzene), is a redox cycling quinone that produces superoxide anions in its free radical state [152]. Paper were published in the 1970s and 1980s [153–157] that attempted to correlate skin-hypersensitivity caused by CDNB administration with cancer prognosis, with the concept of cancer being an autoimmune disease. We could not find any citations after 1987 in this regard. When exposed to CDNB, RM11-1a yeast show a remarkable 1500-fold increase in growth rate as compared to BY4716 yeast in the absence of Hsp90, and a 1500-fold increase in growth rate compared with both RM11-1a and BY4716 yeast in the presence of Hsp90 [69]. This example is illustrative for two reasons, the first being the causative genetic polymorphism maps to the 3' untranslated region of the *NDI1* gene (Fig. 1c, bottom left). The Ndi1 protein encodes an NADH-quinone (Q) oxidoreductase that protects against oxidative stress [158–160]. CDNB produces oxidative stress both by directly producing free radicals, when in its free radical form, and by titrating GSH levels [161–169]. Interestingly, overexpression of Ndi1 increases lifespan in *Drosophila* [170], which is consistent with the free-radical theory of aging [171]. The second reason is that it suggests that Hsp90 functions to regulate NDI1 expression in an indirect rather than a direct manner.

How might Hsp90 affect expression of NDI1 in RM11-1a yeast but not BY4716 yeast? We propose that Hsp90 is a chaperone for a hypothetical 3'UTR binding protein that binds to the NDI1 3'UTR when it has either the RM11-1a or the BY4716 genotype (Fig. 1c,

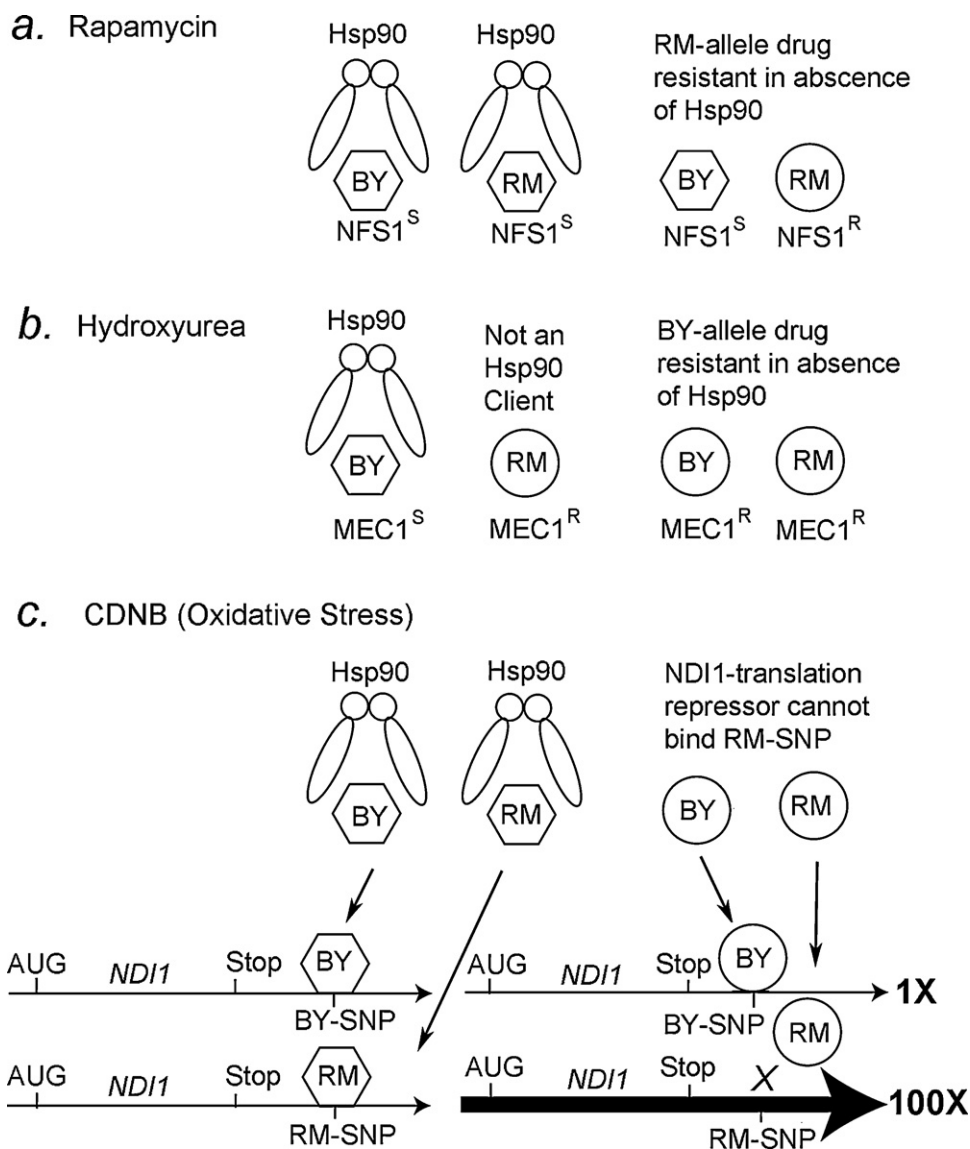


Fig. 1. Hsp90 and drug resistance in yeast. (a) RM11-1a yeast are resistant to rapamycin in the absence of Hsp90. We propose that both BY4716 and RM11-1a Nfs1 proteins are clients for Hsp90 which helps it maintain a rapamycin sensitive phenotype (hexagon), but Nfs1 protein forms a rapamycin-resistant structure (circle) in the absence of Hsp90. (b) BY4716 yeast are sensitive to hydroxyurea in the presence of Hsp90. We propose that the BY4716 Mec1 protein is a client for Hsp90 and it forms a structure that confers hydroxyurea sensitivity (hexagon), but the RM11-1a Mec1 protein is not a client for Hsp90 and forms a structure that confers resistance to hydroxyurea (circle). In the absence of Hsp90, we propose that the BY4716 Mec1 protein folds into a structure that confers resistance to hydroxyurea (circle). (c) RM11-1a yeast are resistant to oxidative stress (CDNB) in the absence of Hsp90. The BY4716 and RM11-1a NDI1 genes have a SNP in the 3'UTR that affects binding to a hypothetical 3'UTR binding protein in the absence of Hsp90 (circle), but not in the presence of Hsp90 (hexagon). See text for more details (see Ref. [69]).

left). In the absence of Hsp90, according to our model, the 3'UTR binding protein folds into a different conformation (a circle) that no longer binds to the NDI1 3'UTR with the RM11-1a genotype, but it can still bind to the NDI1 3'UTR with the BY4716 genotype (Fig. 1c, right). We propose that the 3'UTR binding protein is a translational repressor that also decreases the NDI1 mRNA levels when it is bound. Therefore, the NDI1 gene has much higher expression in RM11-1a yeast compared with BY4716 yeast (Fig. 1c, right, thick arrow). This model would explain why CDNB resistance maps to the 3'UTR of the NDI1 gene and not the hypothetical 3'UTR binding protein.

A fascinating finding of Jarosz and Lindquist is that the clustering of the genotype and the phenotype in 11 different yeast strains is improved in the absence of Hsp90 [69]. Genetic clustering was done by comparing the whole genome sequences of the 11 yeast strains. In the presence of Hsp90, there was no significant clustering of the phenotypes for resistance to 100

different growth conditions, including alternative carbon sources, oxidative stressors, antifungal drugs, small molecule drugs, and DNA damaging agents. However, in the absence of Hsp90, the phenotypes cluster as well as the genotypes. They conclude, "It is difficult to imagine how environmental stress in general, and Hsp90 in particular, could have such a strong impact on genotype-phenotype correlations unless it acted through the evolutionary history of these strains to influence the retention of a broad swath of genetic variation" [69]. In other words, this is the best evidence to suggest that Hsp90 plays a critical role as a capacitor for phenotypic variation, such as in drug resistance in yeast, and probably also drug resistance in cancer. We predict that cancer cell phenotypes, such as growth rates in drug containing media, will cluster with the genotypes better when Hsp90 is inhibited. Understanding this relationship will be needed for facilitating personalized medicine approaches to treating cancer in humans with Hsp90 inhibitors used in combination with other drugs.

5. Summary and future studies

Hsp90 has a unique role in evolution by maintaining the activity of mutant proteins and serving as a capacitor to buffer phenotypic variation [69,71,73,172,173]. The role of Hsp90 in evolution of drug resistance requires study in greater detail. This review collates numerous studies that show that Hsp90 often acts synergistically with other anti-cancer drugs.

Phase II clinical trials of 17-AAG and trastuzumab have shown very promising results [42]. Since Hsp90 has over 200 client proteins, many of which are targeting in treating cancer, it is likely that 17-AAG will be used in combination with many other drugs in future human clinical studies. The classical mechanism for Hsp90 inhibitor function is that the inhibitor causes the degradation of its client proteins. For example, many HER2 positive breast cancers have an over expression of HER2 by gene amplification. In the absence of Hsp90, HER2 cannot be folded properly and is subject to ubiquitin-mediated proteolysis. If there is less HER2, than drugs that target HER2, such as the breast cancer drug trastuzumab is much more effective.

However, the classical mechanism for studying the synergistic action of Hsp90 inhibitors does not take into account the natural genetic variation in the human population. Studies of drug resistance in yeast, which are summarized here, have shown that several anti-cancer drugs are made either more or less potent when in combination with Hsp90 inhibitors, depending on the genetic variation in the yeast. Future studies in humans will need to be done to understand how genetic variation affects drug resistance, and will indicate which drugs will be most effective when used in combination with Hsp90 inhibitors.

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