Genetics

The Role of HSP40 Conserved Motifs in the Response to Cytotoxic Stress

Samantha J. Sojourner, Willie M. Graham, Aurellia M. Whitmore, Jana S. Miles, Devon Freeny, and Hernan Flores-Rozas*

College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA

Doxorubicin, a highly effective therapeutic agent against several types of cancer, is associated with serious side-effects, particularly cardiotoxicity. In addition, drug resistance leads to unsuccessful outcomes in many patients. There are no current biomarkers to indicate doxorubicin treatment response in patients. To understand the mechanisms of toxicity of doxorubicin, a whole-genome sensitivity screen was performed in the yeast *S. cerevisiae*. A deletion mutant of the yeast DNAJ (YDJ1), a J-domain heat-shock protein 40 (HSP40) was among the most sensitive strains. HSP40 is a co-chaperone to HSP70 and together refold denatured proteins into native conformation. The HSP40 YDJ1 is comprised of several highly-conserved domains and motifs that are essential in the heat-shock response. The cysteine-rich region has been implicated in protein-protein interaction with client proteins, farnesylation of YDJ1 facilitates attachment of YDJ1 to the ER and perinuclear membranes, and the histidine-proline-aspartic acid (HPD) tripeptide motif present in the J-domain, is responsible for the regulation of the ATPase activity of HSP70s. We have investigated the role of these motifs in the protection cytotoxic stress. We find that mutations in the HPD motif and cysteine-rich region of YDJ1 sensitize cells to doxorubicin and cisplatin, while a mutation in farnesylation results in a slightly protective effect. The sensitivity of the HPD and cysteine mutants is specific to oxidative stress and not to DNA double-strand breaks.

Cancer | Doxorubicin | Heat shock proteins
Heat-shock response | Reactive oxygen species

Introduction

Doxorubicin is an anthracycline antitumor antibiotic used as first-line treatment, sometimes in conjunction with other chemotherapeutic agents, to treat many types of cancers such as leukemia, lymphoma, soft tissue sarcomas, and multiple myeloma (1). Doxorubicin exerts its anticancer activity through the inhibition of nucleic acid metabolism by intercalation into DNA base pairs and by inhibiting topoisomerase II, thus resulting in the generation of DNA double-strand breaks (DSBs) (2). In addition, doxorubicin increases quinone-type free radical production, which further contributes to cytotoxicity (3). Studies have suggested that free radical-induced DNA damage begins to occur shortly after initiation of doxorubicin treatment (4). The most dangerous side effect of doxorubicin is chronic cardiomyopathy, which can lead to congestive heart failure (5). While the iron-chelating drug dexrazoxane has been used to mitigate doxorubicin's cardiotoxicity by reducing the load of reactive oxygen species in cardiomyocytes (6), it has been associated with the development of secondary cancers including acute myeloid leukemia and myelodysplastic syndrome (7). In addition to severe side effects, the development of drug resistance compromises the efficacy of doxorubicin-based regimens (8).

In an attempt to better understand the underlying mechanisms involved in the sensitivity to doxorubicin, a whole-genome sensitivity screen of the yeast *Saccharomyces cerevisiae* was performed by Ling Xia and colleagues (9). The study characterized several genes and pathways that contribute to protection of the cell from exposure to doxorubicin, including the heat shock response. The *ydj1*- knockout strain was found to be highly sensitive to heat shock and cisplatin exposure in addition to doxorubicin.

YDJ1 encodes for a yeast heat shock protein 40 (HSP40), and is a homolog of the *E. coli* chaperone protein DNAJ. DNAJ, an HSP40, is a crucial factor in the heat shock response (10), the cellular response to certain types of stress such as elevated temperatures, infection, inflammation and starvation. The accumulation of misfolded proteins during certain cellular stresses induces the heat shock response. The response is mediated by genes encoding for heat shock proteins that are up-regulated through transcription (11). Heat shock proteins have essential roles in the folding, unfolding, translocation, and degradation of proteins (12). DNAJs/HSP40s are considered chaperones as they have the ability to bind to exposed hydrophobic residues of both unfolded and nascent polypeptides (13). However, DNAJs/HSP40s are mostly considered to be co-chaperones of DNAKs/HSP70s (14,15). HSP70 functions to refold misfolded polypeptides, but is a weak ATPase and therefore requires stimulation of ATP hydrolysis through its interaction with HSP40. The Ydj1p protein is comprised of several highly-conserved domains/motifs that are essential for its function. The HPD tripeptide motif of the N-terminal J-domain has been demonstrated to regulate the ATPase activity of HSP70s (16–19). The centrally-located cysteine-rich region of YDJ1 has been implicated in protein-protein interaction. Ydj1p is also post-translationally modified by farnesylation which enhances its affinity for the ER and perinuclear membranes of the cell. In this work, we characterize the role of the mentioned motifs of YDJ1 in response to cytotoxic stress. We find that the HPD motif and the cysteine-rich regions are essential for the response to doxorubicin, cisplatin and as previously demonstrated, to heat-shock. The sensitivity of HPD and cysteine mutant strains is, in part, due to doxorubicin-induced oxidative stress and not to the generation of DSBs.

Materials and Methods

Media and chemicals

E. coli strains were grown in LB broth or on LB agar, both supplemented with 100 µg/ml ampicillin (Sigma Aldrich) for plasmid maintenance, when appropriate. Yeast strains were grown

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* Corresponding Author. Hernan Flores-Rozas, Ph.D.
College of Pharmacy and Pharmaceutical Sciences,
Florida A&M University, 1415 S. Martin Luther King Blvd,
Tallahassee, Florida 32307, USA. Phone: 850-412-7088
Email: hernan.floresrozas@famu.edu

in either yeast/peptone/dextrose (YPD) media (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) or Leu- selective media containing 0.67% yeast nitrogen base, 2% agar, 2% glucose (dextrose) or 2% galactose, and 0.087% of a drop-out mix that lacks the amino acid leucine for selection (20,21). When required, etoposide (Chem-Impex Int'l. Inc.) was included in Leu- selective media at 1 mM concentration.

Doxorubicin-HCl (2 mg/mL) was purchased from Bedford Laboratories (Eatontown, NJ, USA); cisplatin (1 mg/mL) was purchased from Calbiochem; menadione (Vitamin K3) was purchased from Enzo Life Sciences (Farmingdale, NY); etoposide was purchased from Chem-Impex Int'l. Inc (Wood Dale, IL).

Nucleic Acid Techniques

Point mutations in each of the motifs studied were introduced into the coding sequence of the *YDJ1* gene cloned in the pBlueScript vector by site-directed mutagenesis. Two overlapping complementary oligonucleotides targeting each motif mutation were synthesized to contain the corresponding nucleotide change(s). Primers were around 50-nucleotides in length. Each primer pair was annealed to the pBlueScript-*YDJ1* clone followed by PCR amplification. After PCR the non-mutated template pBlueScript-*YDJ1* plasmid was eliminated by digestion with DpnI. The reaction mixture was transformed into NEB 5-alpha (New England BioLabs. Ipswich, MA) high efficiency competent cells and colonies were selected on LB-ampicillin plates. Single colonies were isolated, plasmid DNA construct was purified and the mutations introduced into the coding sequences of the YDJ1 motifs were confirmed by DNA sequencing (22). The oligonucleotide primers used for the site-directed mutagenesis are listed in Table 1. The sequence-confirmed pBluescript-YDJ1 mutant constructs were restricted with SacI and EcoRI and the mutant YDJ1 DNA-containing insert was cloned into the pYX243 plasmid vector. Correct constructs were then restricted with SacI and EcoRI to confirm the presence of the mutant YDJ1 DNA insert.

Yeast Genetics

ydj1- strain was grown overnight in YPD media (1% yeast extract, 2% peptone, 2% glucose, 2% agar) at 30°C with shaking, then transformed with pXY243 plasmids harboring mutant YDJ1 using a yeast transformation kit (Frozen EZ Yeast Transformation II, Zymo Research). Transformed cells were selected by plating on Leucine dropout (Leu-) agar glucose plates and grown at 30°C for 2-4 days. Single colonies were picked and glycerol (15% v/v) stocks were stored at -80°C for further analysis (23).

***S. cerevisiae* strains**

The genotypes of all strains used in these studies are shown in Table 2. Homozygous haploid deletion strains library (Parental strain BY4741: *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was obtained from Thermo Fisher Scientific (Pittsburgh, PA). Wildtype *YDJ1* or mutant *YDJ1*-expressing pYX243 plasmids were transformed into the *ydj1-* deletion strain. The *rad52-* (homologous recombination deficient)and *sod1-* (superoxide dismutase deficient) deletion strains, used as controls, were also transformed with pYX243 (2μ, LEU2, Gal-promoter) for appropriate selection.

Table 1: Oligonucleotide Primer Sequences for Site-directed mutagenesis.

Name	Oligonucleotide Set Sequence	Location	Mutation (Amino Acid)
HFR3001	5′ TATAGAAAATGCGCCTTAAAATACCAACCAGATAAGAATCCAAGTGAG 3′(FWD)	HPD motif	T102A (His to Glu)
HFR3002	5′ CTCACTTGGATTCTTATCTGGTTGGTATTTTAAGGCGCATTTTCTATA 3′(REV)		
HFR3003	5′ GGTAAGAAAGGCGCCGTCAAGAAGACTACCAGCTGTAATGGTCAAGGTATT 3′(FWD)	Cysteine repeats	T475A G476C (Cys to Thr)
HFR3004	5′ AATACCTTGACCATTACAGCTGGTAGTCTTCTTGACGGCGCCTTTCTTACC 3′(REV)		
HFR3009	5′ GATGAAGAAGAACAAGGTGGCGAAGGTGTTCAAAGTGCATCTCAATGA 3′ (FWD)	Farnesylation site	T1216A (Cys to Ser)
HFR3010	5′ TCATTGAGATGCACTTTGAACACCTTCGCCACCTTGTTCTTCTTCATC 3′ (REV)		

Table 2: Yeast strains used in this study.

Name	Strain	Genotype
ydj1-	***ydj1-***	*MATa his3-1 leu2Δ met15Δ ura3Δ ydj1Δ [pYX243 LEU2]*
YDJ1	***YDJ1***	*MATa his3-1 leu2Δ met15Δ ura3Δ ydj1Δ [pYX243-YDJ1 LEU2]*
HPD	***ydj1-H34E***	*MATa his3-1 leu2Δ met15Δ ura3Δ ydj1Δ [pYX243-ydj1-H34E LEU2]*
cys	***ydj1-C159T***	*MATa his3-1 leu2Δ met15Δ ura3Δ ydj1Δ [pYX243-ydj1-C159T LEU2]*
far	***ydj1-C406S***	*MATa his3-1 leu2Δ met15Δ ura3Δ ydj1Δ [pYX243-ydj1-C406S LEU2]*
rad52-	***rad52-***	*MATa his3-1 leu2Δ met15Δ ura3Δ rad52Δ [pYX243 LEU2]*
sod1-	***sod1-***	*MATa his3-1 leu2Δ met15Δ ura3Δ sod1Δ [pYX243 LEU2]*

Table 3: Sensitivity of YDJ1 strains to cytotoxic stressors.

Strain	Heat Shock		Doxorubicin		Cisplatin	
	Survival (% ± SEM)	Sensitivity (fold)	Survival (% ± SEM)	Sensitivity (fold)	Survival (% ± SEM)	Sensitivity (fold)
ydj1	0 ± 0.0	---	2 ± 1.3	34.0	1 ± 0.1	86.0
YDJ1	91 ± 11.1	1	68 ± 4.3	1.0	86 ± 21.0	1.0
HPD	2 ± 2.1	45.5	8 ± 3.8	8.5	7 ± 3.0	12.3
cys	2 ± 1.6	45.5	3 ± 1.0	22.7	2 ± 0.5	43.0
far	85 ± 8.6	1.1	81 ± 3.8	0.8	115 ± 8.1	0.7
rad52					3 ± 0.8	28.7

Cytotoxic stress sensitivity assays

The concentrations of drug and lengths of exposure to heat shock (37°C), doxorubicin, cisplatin, menadione and etoposide were previously determined experimentally using the wild type parental strain, BY4741, as well as the mutants *ydj1-* strain. Colonies of each strain containing a YDJ1 mutation were selected on Leu- agar, glucose plates and grown overnight (~20 hours) in Leu-, galactose (2%), glucose (0.5%) liquid medium at 30°C with shaking. Following overnight culturing, cells were washed and re-suspended in sterile water. Cells suspensions were separated into control (no drug, or 30°C for heat shock test) and test groups, where cells were treated by exposure to various chemical agents for a period of 30 minutes (for doxorubicin), or 3 hours (cisplatin and menadione). After the period of exposure, cells were once again washed and suspended in sterile water. Serial dilutions were performed on 96-well plates, and 10 µl of cells from each dilution was spotted onto Leu- agar, galactose plates. Plates were incubated at 30°C (heat shock treated cells were incubated at 37°C) for a period of 72 hours. Cells treated with etoposide were washed, serially diluted, and spotted directly onto Leu-, galactose, etoposide plates to grow for 72 hours at 30°C. Cell growth was monitored daily, and strain sensitivity was scored after 72 hours by calculating survival using the *ydj1-* +pYX243[YDJ1] strain as the control. Sensitivity was calculated relative to untreated controls. Data was collected from at least 3 trials for each YDJ1 mutant and for each stressor. Survival percentages were calculated by dividing the number of colonies present on treated plates by the number of colonies present on non-treated plates for each strain.

Statistical Analysis

Data analysis and graphing was performed using Graph Pad Prism 6 software. Specific analysis for each experiment is indicated in each figure legend. The mean of at least three experiments is plotted together with the standard error of the mean. Significance was determined using One-way ANOVA.

Results

Differential sensitivity of *YDJ1* mutants to Heat Shock, Doxorubicin and Cisplatin

All pYX243-based constructs were generated and confirmed as described in the Materials and Methods. The mutations introduced in the *YDJ1* gene are described in Table 1. Plasmids harboring these gene alleles were transformed into the *ydj1-* strain as described in the Materials and Methods. The strains generated and used for this study are described in Table 2. To observe the sensitivity of *YDJ1* mutant strains to cytotoxic stress, strains were exposed to heat shock, doxorubicin, and cisplatin as described in the Materials and Methods. Figure 1 displays results from exposure of *YDJ1* strains to heat shock. As observed in Figure 1A, the *ydj1-* deletion strain, which was included as a positive control for sensitivity, does not survive after exposure to heat shock as previously described. The strain expressing wild type YDJ1 (Fig. 1A, YDJ1), survives heat shock treatment, with a 91% survival. Analysis of the strains harboring *YDJ1* mutant alleles indicates that the cysteine and HPD mutant strains displayed sensitivity comparable to that of the *ydj1-* strain (2%, 2% and 0%, respectively), while the farnesylation mutant strain displayed survival (85%) comparable to the strain complemented with the wild type YDJ1 strain. Quantitative analysis is displayed in the Fig. 1B, which illustrates the mean survival (in percentage) in response to heat shock (N=3) of the YDJ1 strains relative to the untreated control (Fig 1A). The data indicate that cys and HPD mutants displayed a 45-fold increased sensitivity ($p<0.05$) to heat-shock relative to the YDJ1-complemented strain (Table 3).

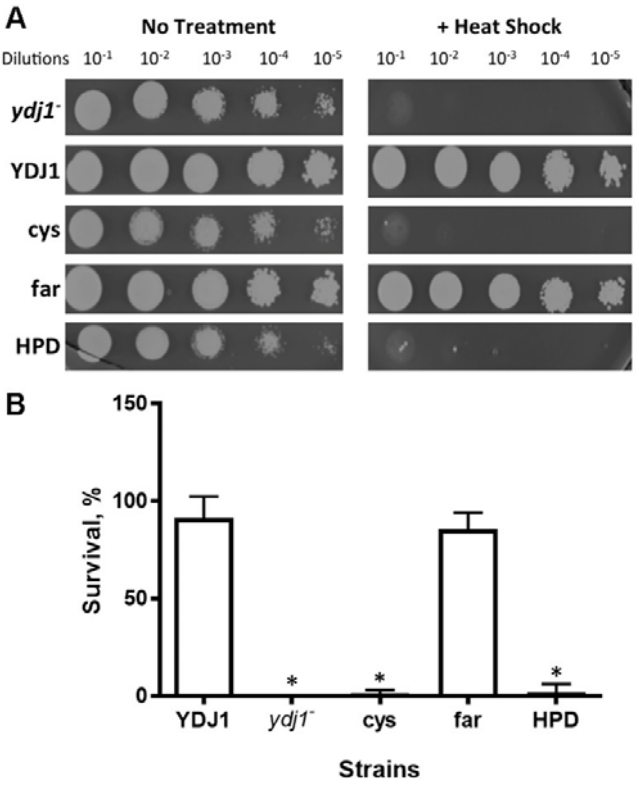

Figure 1. Sensitivity of YDJ1 mutants to heat shock. A. Growth of YDJ1 mutants in response to heat shock. Strains growth tested by spotting onto Leu- agar, galactose plates. **B.** Survival rates of strains following heat shock. Survival was compared to the YDJ1-complemented strain. * indicates p-values less than 0.05 which relate to strong presumption of statistical significance.

The YDJ1 strains were tested for sensitivity to doxorubicin exposure (Fig 2A). The *ydj1-* deletion strain is notably sensitive, as growth is significantly repressed (2% survival) compared to the complemented YDJ1 strain (68% survival), at the concentration of doxorubicin tested. The cyst and HPD mutant strains also displayed repressed growth similar to the *ydj1-* strain (3% and 8% survival, respectively). Inversely, farnesylation mutant strain was resistant to doxorubicin exposure, displaying slightly more resistance than YDJ1 (81% vs 68% survival). Figure 2B illustrates the mean survival percentage of the YDJ1 strains following doxorubicin exposure (N=3). Table 3 displays the fold sensitivity of each strain compared to the positive control strain (YJD1). The cysteine mutant displayed a ~23-fold increased sensitivity, while the HPD mutant displayed an 8-fold increased sensitivity to doxorubicin relative to the YDJ1-complemented strain (Table 3). The differences in response of the *ydj1-* deletion, cysteine, and HPD mutants to doxorubicin compared to the positive control are statistically significant ($p<0.05$), while there is not much difference between the survival of the farnesylation mutant and the positive control YDJ1.

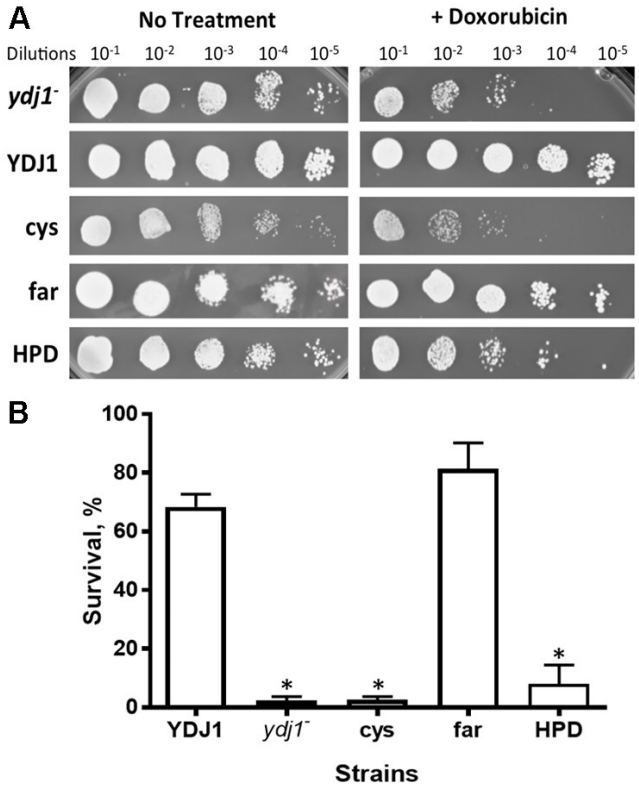

Figure 2. Sensitivity of YDJ1 mutants to doxorubicin. A. Growth of YDJ1 mutants in response to doxorubicin exposure. Strains growth tested by spotting onto Leu- agar, galactose plates. **B.** Survival rates of strains following doxorubicin exposure. Survival of mutant alleles was compared to the YDJ1-complemented strain. * indicates p-values less than 0.05 which relate to strong presumption of statistical significance.

Responses of the YDJ1 strains to cisplatin exposure are shown below (Fig. 3). The positive control used in cisplatin sensitivity assays was *rad52-*, a deletion strain deficient in the gene encoding RAD52, which is involved in DNA double-strand break repair by homologous recombination. Because cisplatin causes DNA cross-linking, it is expected that cells lacking the ability to repair DNA damage would be sensitive to cisplatin exposure. The YDJ1 strains displayed similar responses to doxorubicin exposure. The *ydj1-* and *rad52-* strains were sensitive to cisplatin as expected (1% and 3% survival, respectively). Similarly, the cysteine and HPD strains also displayed high sensitivity to cisplatin (2% and 7% survival, respectively). The YDJ1 and farnesylation strains showed almost no inhibition of growth at the concentration of cisplatin tested. Figure 3B illustrates the mean of the survival (in percentage) of the strains following exposure to cisplatin (N=3). Table 3 displays the fold sensitivity of each strain compared to the positive control strain (YJD1). The sensitivity to cisplatin varied depending on the mutant, with the cysteine mutant being 43-fold and the HPD ~12-fold more sensitive than the YDJ1-complemented strain. By comparison, the *ydj1-* strain was 86-fold more sensitive than the YDJ1-complemented strain (Fig 3B and Table 3).

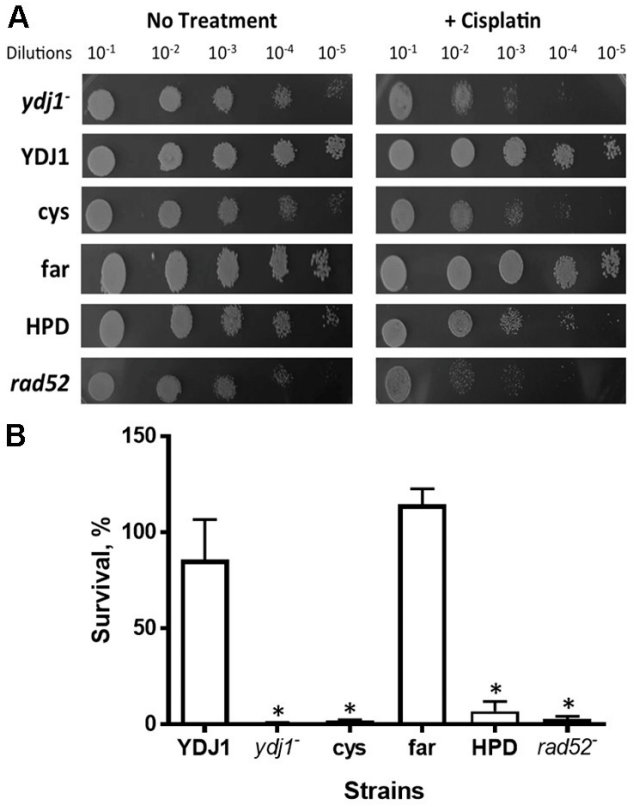

Figure 3. Sensitivity of YDJ1 mutants to cisplatin. A. Growth of YDJ1 mutants in response to cisplatin treatment. Colony growth of strains spotted onto Leu- agar galactose plates. **B.** Survival rates of strains following cisplatin exposure. * indicates p-values less than 0.05 which relate to strong presumption of statistical significance.

Sensitivity of YDJ1 mutants to etoposide and menadione

To identify the specific toxic mechanism to which the *YDJ1-* mutants strains are sensitive to doxorubicin, they were exposed to etoposide, which generates DSBs and menadione, which generates reactive oxygen species (ROS).

Sensitivity of YDJ1 strains to etoposide are shown below (Fig. 4). The *rad52-* deletion strain was also used as a positive (sensitive) control for etoposide treatment. Etoposide inhibits topoisomerase II in same manner as doxorubicin, resulting in DNA double strand breaks, however, it does not generate ROS. It would be expected that cells deficient in the RAD52 would be sensitive to etoposide, as RAD52 is an important protein for DNA double-strand break repair. In fact, the *rad52-* displayed a 20-fold increased sensitivity (5% survival) to etoposide relative to the YDJ1-complemented strain (Fig 4B and Table 4). However, the

YDJ1 mutant strains did not display sensitivity to etoposide exposure. The *ydj1-* mutant strain along with the cysteine, HPD, and farnesylation strains displayed survival similar to the complemented YDJ1 strain. Figure 4B illustrates the mean survival percentages of the YDJ1 strains following exposure to etoposide (N=3). Table 4 displays the fold sensitivity of each strain compared to the wild type YDJ1-complemented strain (YJD1). Only the positive control *rad52-* was sensitive to etoposide (5% survival).

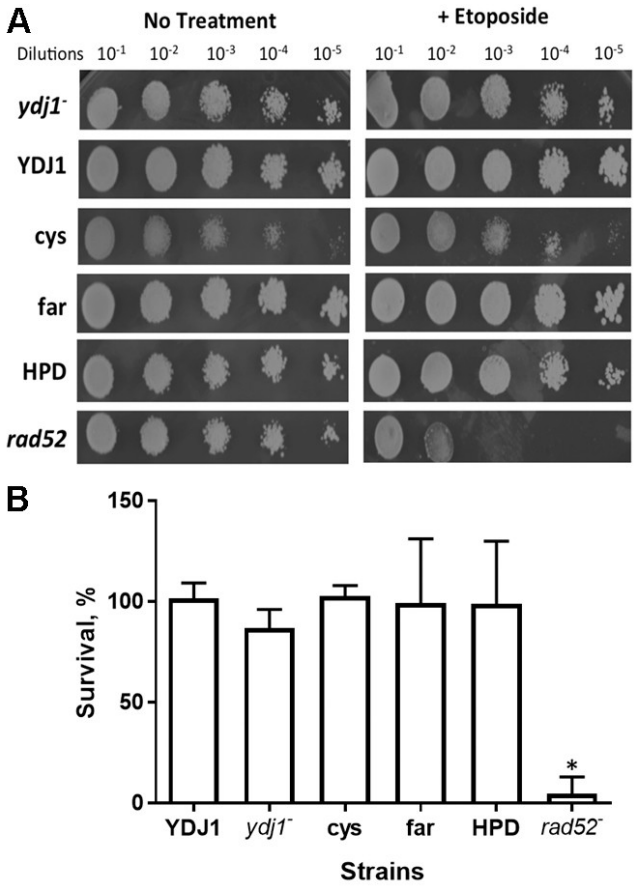

Figure 4. Sensitivity of YDJ1 mutants to etoposide. A. Growth of YDJ1 mutants in response to etoposide. Growth of different YDJ1-alleles strains was tested by spotting onto Leu- agar galactose plates. **B.** Survival rates of strains following etoposide treatment in percentage. * indicates p-values less than 0.05 which relate to strong presumption of statistical significance.

Sensitivity of YDJ1 strains to menadione is shown below (Fig. 5). The *sod1-* deletion strain was used as the positive control for sensitivity to menadione treatment. Menadione features the same quinone ring present in the doxorubicin structure, both of which produce quinone-type free radicals including superoxide. SOD1 is a superoxide dismutase that destroys free superoxide radicals. Without SOD1, it is expected that the cell would not be able to protect itself from agents that produce superoxide. The response of the strains was similar to that observed for doxorubicin and cisplatin responses. While *sod1-* was sensitive to menadione exposure (no growth, Fig 5A and 5B), as expected, *ydj1-* deletion strain, cysteine mutant, and HPD mutant were also sensitive to menadione (1%, 3% and 11% survival, respectively). YDJ1 and farnesylation were resistant (85% survival). Figure 5B illustrates the mean survival percentages of the YDJ1 strains following exposure to etoposide (N=3). Table 4 displays the fold sensitivity of each strain compared to the wild type YDJ1-complemented strain (YJD1). The *ydj1-* and the cys mutants displayed significant sensitivity (57- and 19-fold, respectively. $p<0.05$), while the HPD mutant was ~5-fold more sensitive than the YDJ1-complemented strain.

Table 4: Sensitivity of YDJ1 strains to etoposide and menadione

Strain	Etoposide		Menadione	
	Survival (% ± SEM)	Sensitivity (fold)	Survival (% ± SEM)	Sensitivity (fold)
ydj1-	87 ± 5.4	1.2	1 ± 0.8	57
YDJ1	102 ± 4.4	1	57 ± 1.8	1
HPD	99 ± 18.0	1	11 ± 4.6	5.18
cys	103 ± 3.1	1	3 ± 1.1	19
far	99 ± 18.0	1	85 ± 9.6	0.7
rad52-	5 ± 4.8	20.4		
sod1-			0 ± 0.0	---

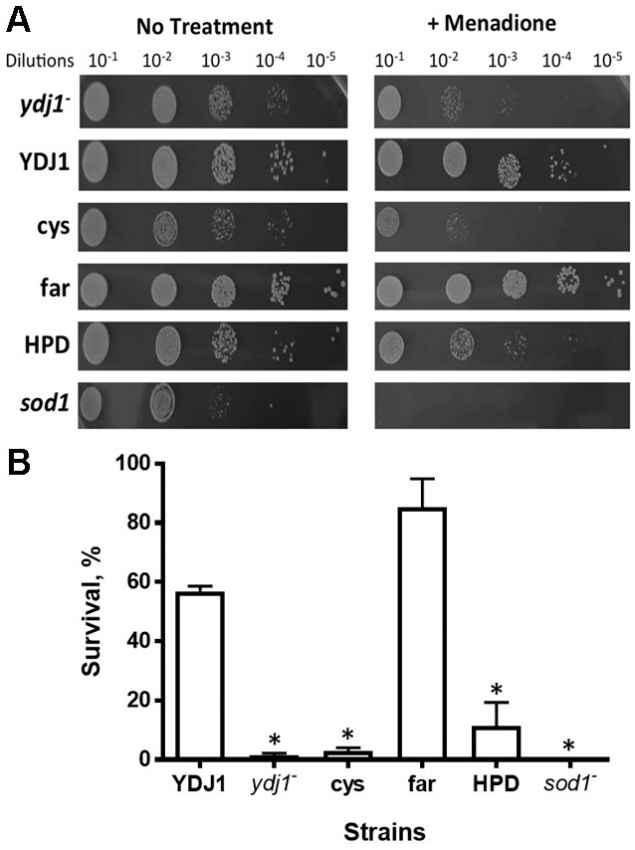

Figure 5. Sensitivity of YDJ1 mutants to menadione. A. Growth of YDJ1 mutants in response to menadione exposure. Colony growth of strains spotted onto Leu- agar galactose plates. **B.** Survival rates of strains following menadione treatment. * indicates p-values less than 0.05 which relate to strong presumption of statistical significance.

Discussion

Each of the targeted motifs has been previously mentioned to be essential for the function of YDJ1 in the heat shock response. The importance of these motifs has not been previously evaluated in response to chemotherapeutic drugs exposure. Strains with mutations in each of the targeted motifs were created through point mutations introduced into the YDJ1 gene by site-directed mutagenesis and cloned into the inducible expression plasmid pYX243, followed by transformation into *ydj1-* strains. Resulting

strains had disruptions in either the HPD tripeptide motif, cysteine-rich region, farnesylation sites of the YDJ1 protein. Mutations in some of these motifs altered the function of YDJ1 in response to cytotoxic stress.

Identifying motifs of YDJ1 that are essential for cellular protection against cytotoxic stress may result in the identification of new targets for mitigating drug resistance and negative effects of doxorubicin treatment.

We determined that mutations in the HPD motif and cysteine-rich region of YDJ1 resulted in strains with a sensitive phenotype that could not survive upon exposure to heat shock, doxorubicin, and cisplatin. These findings imply that a correctly functioning HPD motif and cysteine-rich region are necessary for proper function of YDJ1 during the response to cytotoxic stressors. The HPD and cys mutant strains were several-fold more sensitive to the above-mentioned treatments compared to YDJ1, which served as the positive control, as the wild type YDJ1 gene without any mutations was transformed into this strain.

The J-domain of YDJ1 harboring the HPD motif is believed to be the major site of interaction with HSP70. The ATP-binding form of HSP70 has a low affinity for unfolded peptides. In the presence of HSP40, however, ATP hydrolysis is enhanced. This leads to enhanced HSP70 activity. Mutations in the HPD motif have been shown to eliminate stimulation of HSP70 ATPase activity (24).

YDJ1 contains a conserved zinc finger-like region that includes 4 repeats of the CXXCXGXG motif, where C is cysteine and G is glycine. The repeats function in pairs to form a zinc-binding domain, which are implicated in protein-protein interactions (25,26). Mutations in the zinc finger-like region have been shown to reduce the activity of type 1 HSP40s (27,28). Fan *et al*. found that mutations in the zinc finger-like region of YDJ1 resulted in defects in androgen receptor function and luciferase refolding. They found that the zinc finger-like region of type 1 HSP40s is required for HSP70 capture of non-native polypeptides from YDJ1 (29).

While the responses of the HPD and cys mutant strains were consistent with findings in the literature and their essential role in the heat shock response, the farnesylation site mutant strain was not observed to be sensitive to any of the stressors used in this study. In fact, mutation of the farnesylation site of YDJ1 caused the strain to become more resistant than the positive control YDJ1 strain after exposure to doxorubicin and cisplatin. The farnesylation mutant strain had survival rates similar to the YDJ1 control strain in response to heat shock and etoposide.

Type 1 HSP40s contain a unique CAAX box at the C-terminal of the protein which becomes post-translationally modified with a farnesyl group (30,31). Farnesylation of type 1 HSP40s aids in localizing the protein to the ER membrane, where they function to fold polytopic membrane proteins. Since all YDJ1 proteins are farnesylated, but many are found in the cytosol, it is thought that farnesylation may have additional roles. Although the significance of YDJ1 farnesylation in the heat shock response has not been extensively studied, it has been shown to be essential for YDJ1 protection against prion toxicity (32).

The absence of sensitivity of farnesylation mutant strains to cytotoxic stressors is logical if farnesylation of YDJ1 is not necessary for proper function in the heat shock response. However, it is not known why a mutation in the farnesylation site of YDJ1 leads to an increase in resistance to cytotoxic stress. One theory is that the loss of YDJ1 farnesylation results in loss of ER membrane sequestration of the protein, allowing for increased overall interaction with HSP70 and client proteins in the cytoplasm.

The response of all strains to doxorubicin and cisplatin was almost identical, suggesting that the studied YDJ1 motifs may be necessary for cell survival in response to diverse cytotoxic agents. In addition, strains responded similarly to heat shock exposure as to doxorubicin and cisplatin, suggesting that the mechanism of protection by HSP40 against cytotoxic agents is similar to that of heat shock.

Etoposide and menadione were included in this study to elucidate the cytotoxic mechanism of doxorubicin in the role of a gene encoding a factor involved in protein folding. Doxorubicin is known to exerts toxic effects through poisoning of topoisomerase II leading to DSBs and the formation of ROS through its quinone ring. Etoposide acts through topoisomerase II inhibition like doxorubicin, and menadione contains the same quinone ring as doxorubicin and can produce quinone-type free radicals. It was observed that *YDJ1* mutant strains responded similarly to menadione as to doxorubicin and cisplatin; the HPD and cys mutants were sensitive, and the YDJ1 control and farnesylation mutant, resistant. However, none of the YDJ1 mutant strains were sensitive to etoposide, only the positive control *rad52* deletion strain was sensitive. These results suggest that the production of ROS, such as superoxide, may the cause of protein damage in the cell. While DNA double-strand breaks affects the cell on the DNA level, these damages do not require a functional YDJ1. In contrast, proteins can be damaged by reactive oxygen species through direct oxidation of amino acid residues and cofactors (33). Cisplatin can also bind to protein, and possibly affect their structure by forming adducts, explaining the requirement for protein chaperones in cell survival. In addition, cisplatin has the potential of forming DNA-protein crosslinks (34).

Yeast strains containing mutations in the conserved HPD motif and cysteine-rich region of YDJ1 display distinct sensitivity when exposed to chemotherapeutic agents as well as heat shock, suggesting that they play essential roles in protection of the cell from cytotoxic stress. Targeting these regions of the YDJ1 protein may provide a useful method of sensitizing cancer cells to chemotherapy, lowering the effective therapeutic dose and concomitantly resulting in reduction of side effects.

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