Chemogenomic screening identifies the Hsp70 co-chaperone DNAJA1 as a hub for anticancer drug resistance

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Heat shock protein 70 (Hsp70) is an important molecular chaperone that regulates oncoprotein stability and tumorigenesis. However, attempts to develop anti-chaperone drugs targeting molecules such as Hsp70 have been hampered by toxicity issues. Hsp70 is regulated by a suite of co-chaperone molecules that bring “clients” to the primary chaperone for efficient folding. Rather than targeting Hsp70 itself, here we have examined the feasibility of inhibiting the Hsp70 co-chaperone DNAJA1 as a novel anticancer strategy. We found DNAJA1 to be upregulated in a variety of cancers, suggesting a role in malignancy. To confirm this role, we screened the NIH Approved Oncology collection for chemical-genetic interactions with loss of DNAJA1 in cancer. 41 compounds showed strong synergy with DNAJA1 loss, whereas 18 dramatically lost potency. Several hits were validated using a DNAJA1 inhibitor (116-9e) in castration-resistant prostate cancer cell (CRPC) and spheroid models. Taken together, these results confirm that DNAJA1 is a hub for anticancer drug resistance and that DNAJA1 inhibition is a potent strategy to sensitize cancer cells to current and future therapeutics. The large change in drug efficacy linked to DNAJA1 suggests a personalized medicine approach where tumor DNAJA1 status may be used to optimize therapeutic strategy.

Hsp70 is a molecular chaperone that plays important roles in protein quality control processes such as protein folding, transport, degradation, and the prevention of protein aggregation. Hsp70 levels are elevated in various cancers and overexpression correlates with poor prognosis for survival and response to cancer therapy. The elevated levels of Hsp90 and Hsp70 chaperones in cancer and their role in fostering multiple oncogenic pathways has made these proteins attractive drug targets with numerous anti-chaperone compounds having been developed so far. Problematically, Hsp70 is required for cell survival and protein homeostasis, and thus its inhibition is detrimental to the viability of both normal and cancer cells, with dubious selectivity for tumor cells.

Hsp70 performs all its functions in association with a large spectrum of helper proteins known as co-chaperones that include J-proteins, tetratricopeptide repeat (TPR) domain-containing proteins and nucleotide exchange factors (NEFs) which fine-tune Hsp70 specificity and activity in the cell. The J-proteins recruit the protein substrates or clients and interact with such clients at the interface of NBD and SBDβ of Hsp70. This interaction leads to increased Hsp70-mediated ATP turnover and activation of protein folding. J-proteins have a highly conserved 70 amino acid motif containing Histidine, Proline and Aspartic acid amino acid residues known as HPD motif which is essential for stimulating ATPase activity of Hsp70. In humans, the J-protein family has about 50 members which are further divided into three groups based on the localization of J-domain within a protein. The Hsp40 DNAJA1 (more commonly referred to as DNAJA1) associates with unfolded polypeptide chains, preventing their aggregation. Several Hsp70 inhibitors have failed in clinical trials due to their toxicity. More recently, alternative strategies have focused on sensitizing cells to anticancer agents by either manipulating post-translational modification of chaperones or their interaction with specific co-chaperones.

DNAJA1 (mammalian homolog of yeast Ydj1) is an interesting possible anticancer target as a key mediator of Hsp70 function that appears to regulate specific features of tumorigenesis. A recent study demonstrated that CRPCs expressing ARv7 are insensitive to Hsp90 inhibitors but are sensitive to Hsp40 inhibition. In addition, we have shown that targeting specific oncoprotein complexes (ribonucleotide reductase) with a combination of...
DNAJA1 is mutated and overexpressed in a variety of cancers. While the roles of Hsp90 and Hsp70 in cancer have been thoroughly studied, much less is known of the role that regulatory co-chaperone proteins such as DNAJA1 play in tumorigenesis. As a first step, we queried the cBioPortal cancer genomic database (cbioportal.org) to determine the incidence of DNAJA1 alterations in cancer. Analysis of data from 176 non-redundant studies representing 44,347 patient samples revealed that DNAJA1 was altered at a frequency of greater than 1% in 35 cancer types (Fig. 1A). Although the majority of alterations in DNAJA1 occur at a relatively low frequency (<5% of cancers) DNAJA1 is significantly amplified in prostate neuroendocrine cancer (PNC) and castration-resistant prostate cancer at a frequency of 17.31% and 17.14% respectively (Fig. 1A). Hsp70 and Hsp90 are often overexpressed in tumors. To determine whether the DNAJA1 expression is also overexpressed in cancer, we analyzed DNAJA1 mRNA expression in samples from the TCGA-PAN-CAN Atlas. Interestingly, DNAJA1 mRNA was expressed at significantly higher levels in these samples, with a median expression in cancer over 3,000 × relative to WT reference samples (Fig. 1B). To determine if this dramatic overexpression of DNAJA1 was a result of amplification, we plotted DNAJA1 expression vs amplification (Fig. 1C). Interestingly, there was minimal correlation between amount of amplification and DNAJA1 expression (r = 0.45) suggesting that while DNAJA1 may be an important marker in cancer it is not caused by gene amplification.

Characterizing the role of DNAJA1 in anticancer drug resistance. The existing literature is contradictory as to whether DNAJA1 may possess tumor suppressor or driver properties. To clarify whether silencing of DNAJA1 could be beneficial in the treatment of cancer, we screened wildtype HAP1 cells and HAP1 cells lacking DNAJA1 (HAP1ΔDNAJA1 KO) for comparative resistance against the NIH NCI Approved Oncology Collection (Fig. 2A) (https://dtp.cancer.gov/organization/dscb/obtaining/available_plates.html). Prior to screening, we validated the status of the DNAJA1 knockout cell line by Western blotting for DNAJA1 and other major chaperones and co-chaperones (Hsp70, Hsc70, Hsp90, Bag-3 and Hsp110). As expected, we confirmed loss of DNAJA1 ability to demonstrate any compensatory effects on the levels of the other chaperones/co-chaperones studied (Fig. S1). According to pharmacologic action, the compounds in the library have been divided into seven categories: protein synthesis inhibitors, proteasome inhibitors, epigenetic modifiers, metabolic inhibitors, cytoskeletal inhibitors, signal transduction inhibitors and DNA synthesis/repair inhibitors. Further fold enrichment of each drug category was calculated for the drugs whose potency increased or decreased with loss of DNAJA1. To monitor the screening quality, each screening plate contained control wells treated with vehicle (1% DMSO). The final concentration of the screening compounds was 50 μmol/L. Positive hits (synergistic) or inhibitors and proteasome inhibitors (Fig. 2E). For a full list of drugs in each category and raw data from screen, please see supplemental Table T1.

Strikingly, a small number of compounds with supposedly related function showed dissimilar alteration of potency upon loss of DNAJA1 function, potentially caused by off-target drug effects (see discussion).
Validation of anticancer drugs significantly altered for potency upon loss of DNAJA1. Many anticancer compounds have low potency, poor therapeutic index or suffer from the development of resistance. Monotherapy is rarely efficient and instead drug cocktails are widely used in the clinic. Establishing these combinations can enhance the scope of preclinical studies and inform the design of future clinical trials. Although knockout of DNAJA1 substantially increased the potency of a number of anticancer molecules, it remained to be determined whether small-molecule inhibition of DNAJA1 could produce a similar result. Our previous bioinformatics analysis indicated that a large proportion of prostate cancer cells contain either amplification or mutation of DNAJA1 (approximately 18%, see Fig. 1). To validate the results of our initial screen, we analyzed the effect of treating prostate cancer cells (LNCaP) with a combination of 116-9e, a small molecule inhibitor of DNAJA1 and selected hits from our screen. We decided to focus on three synergistic drugs discovered in the screen: cabozantinib (receptor tyrosine kinase inhibitor), clofarabine (an RNR inhibitor) and vinblastine (microtubule inhibitor/G2 arresting agent). We also validated three drugs that demonstrated a significant loss of potency in cells lacking DNAJA1: sorafenib (a VEGFR-2 inhibitor), omacetaxine mepesuccinate (more commonly known as homoharringtonine, a protein translation inhibitor) and idarubicin (topoisomerase II inhibitor). To determine synergy in a quantitative manner, we calculated drug synergy (Combination Index values, CI) between 116-9e and either synergistic or antagonistic drugs hits across a broad range of concentrations using the Chou-Talalay method (for effects of individual drugs, please see Fig. S2). For three hits identified in our screen (cabozantinib, clofarabine and vinblastine) we confirmed significant synergy (CI < 1) between 116-9e and either synergistic or antagonistic drugs hits across a broad range of concentrations using the Chou-Talalay method (for effects of individual drugs, please see Fig. S2). For three hits identified in our screen (cabozantinib, clofarabine and vinblastine) we confirmed significant synergy (CI < 1) between 116-9e and either synergistic or antagonistic drugs hits across a broad range of concentrations using the Chou-Talalay method (Figs. 3A–C). In contrast, idarubicin, omacetaxine and sorafenib displayed a significantly antagonistic interaction (CI > 1) across a range of doses (Fig. 3D–F). These data suggest that while DNAJA1 inhibition is a promising strategy to sensitize cells to a number of currently used anticancer drugs, the loss of DNAJA1 can significantly decrease the potency of a small subset of inhibitors.

Evaluating the effects of dual targeting of identified drugs with DNAJA1 inhibition on morphology and viability of prostate cancer spheroids. Recent studies have suggested that precision
therapy approaches involving the exposure of drugs directly to the primary tumor tissue have the potential to augment the personalized medicine efforts and influence clinical decisions. Establishing ex vivo three-dimensional (3D) tumor spheroids or organoids derived from primary cancers can be easily established and potentially scaled to screen drug combinations. These 3D cancer models appear to recapitulate features of the tumor of origin in terms of heterogeneity, cell differentiation, histoarchitecture, and clinical drug response and can be used for rapid drug screening. We therefore next examined the effect of drug combination (three antagonistic and synergistic hits) on LNCaP spheroids. Specifically, changes in spheroid size and shape induced by the 3 antagonistic and synergistic drugs were determined. Visual examination revealed that for the synergistic drugs combination with 116-9e resulted in physical disruption of LNCaP spheroids, resulting in decrease in spheroid size (Fig. 4A). The disruption started on the second day of the treatment. However, when the 3 antagonistic drugs were administered along with 116-9e, there were minimal changes in spheroid morphology indicating that the combination was ineffective.

Next, we measured the induction of apoptosis in the spheroids post drug treatments. We determined the kinetics of apoptosis induction using AnnexinV/PI staining. Drug-induced apoptosis was readily detected in the LNCaP spheroids treated with mono and dual drug combinations. In concurrence with the previous results, the combination of the three synergistic drugs with 116-9e displayed enhanced apoptosis as compared to the single drug treatment whereas spheroids treated with the 3 antagonistic drugs showed little or no difference in the rate of apoptosis as compared to the dual drug combination with 116-9e (Fig. 4B).
Discussion

Although inhibitors of Hsp70 and Hsp90 have been developed for research purposes, the conversion of these molecules for use in patient treatment have been hampered by toxicity issues. We undertook this study to resolve conflicting literature on whether inhibiting DNAJA1, a co-chaperone of Hsp70 may be useful as a novel anticancer strategy. Our bioinformatic analysis of DNAJA1 expression and mutation clearly identify DNAJA1 as being highly altered in a range of cancers, particularly in prostate cancer. Interestingly, DNAJA1 despite being substantially overexpressed in a range of cancers, there was minimal correlation between DNAJA1 copy number and level of expression. While beyond the scope of this study, it is possible that the high levels of DNAJA1 expression observed may be a result of increased transcription brought on hyperactive signaling pathways common in cancer cells. This data in conjunction with a recent finding that Hsp40 is involved in regulation of ARv13 and p5328 makes DNAJA1 inhibition an ideal choice as a novel therapeutic target in Prostate Cancer.

In this study, loss of DNAJA1 increased the potency of a substantial number (31%) of clinically used anticancer drugs. This increased potency may be related to the destabilization of clients that are the target of these small molecules. For example, Hsp70 activates many proteins involved in the DNA damage response and DNA repair pathways (DDR), including ATM, APE1, PARP1, XRCC129. Recently, studies from my group have established roles for both Hsp70 and DNAJA1 in stability of the RNR complex8,29,30. It is unsurprising then that many of the anticancer agents displaying synergy with loss of DNAJA1 are connected to inhibition of the DNA damage pathway.

Figure 3. Drug interaction between 116-9e (DNAJA1 inhibitor) and selected hits. LNCaP cells were treated with different concentrations of cabozantinib (A), clofarabine (B), vinblastine (C), idarubicin (D), omacetaxine (E) and sorafenib (F) with or without 116-9e for 72 h in RPMI-1640 medium containing 10% FBS. Each point is the mean ± SD for three independent experiments. Growth inhibition was determined using Cell Titer-Glo assay. Combination Index (CI, measure of drug synergy) was determined using Chou-Talalay method via Compusyn software. CI values are as follows: <0.1 (very strongly synergistic), 0.1–0.3 (strongly synergistic), <0.9 (synergistic), 0.9–1.1 (additive), 1.1–3.3 (antagonistic), 3.3–10 (strongly antagonistic), >10 (very strongly antagonistic).
response/repair. These include molecules such as 5-fluorouracil (5-FU), premetrexed, clofarabine, olaparib and niraparib etoposide, teniposide and valrubicin. Here we validated synergy with the RNR inhibitor clofarabine. Clofarabine is phosphorylated intracellularly to form cytotoxic active 5′-triphosphate metabolite, which inhibits the enzymatic activities of RNR and DNA polymerase, resulting in inhibition of DNA synthesis and repair.

While most DDR inhibitors displayed increased potency with DNAJA1 depletion, four of them were antagonistic to loss of DNAJA1. These include topoisomerase inhibitors and nucleic acid synthesis inhibitors such as trifluridine, irinotecan, epirubicin (4′-epi-isomer of the antibiotic doxorubicin) and idarubicin (4-demethoxy analogue of daunorubicin). While at first these results seem paradoxical, it is worth noting that inrinotecan is a type I topoisomerase inhibitor, whereas Etoposide (synergistic with loss of DNAJA1) is a type II topoisomerase inhibitor. It may be that Hsp70 and DNAJA1 play opposing regulatory roles in the stabilization and activation of these related proteins.

In addition to DDR, DNAJA1 is also involved in signal transduction, with previous reports indicating that the yeast homolog of DNAJA1 (Ydj1) is critical for supporting the integrity of kinase signaling networks. DNAJA1 is mobilized to specific sites within the nucleus in response to inappropriate targeting or folding of specific mutant receptors. DNAJA1 overexpression ameliorates the defective transactivation and trans-repression activity of mutant Glucocorticoid receptors.

In line with the previous studies, we found that a handful of Receptor Tyrosine kinase inhibitors were synergistic with DNAJA1 depletion. These included Vascular endothelial growth factor receptor (VEGFR) inhibitors such as sunitinib, cabozantinib, lenvatinib and pazopanib. Interestingly, randomized phase III clinical trials are being conducted to validate the efficacy of Cabozantinib in heavily pretreated prostate cancer patients.

One implication from our study is that DNAJA1 inhibition might significantly enhance the effect of cabozantinib monotherapy.

Strikingly, some of the kinase inhibitors were antagonistic to DNAJA1 depletion. These include VEGFR inhibitors such as regorafenib and sorafenib. This disparity can be explained by the different target receptors and mechanisms of action of these drugs. Interestingly, recent studies indicated that these small molecule inhibitors exhibit off-target effects. Some of these drugs are misidentified and mischaracterized for their target specific inhibition, which has contributed to the high failure rate of these drugs in the treatment of cancer patients.

In addition to its role in signal transduction, DNAJA1 is also important for maintaining the cellular cytoskeleton. Previous studies have suggested that Ydj1 (the yeast homolog of DNAJA1) is important for the proper assembly of microtubules. Another report showed that DNAJA1 depletion causes relocation of N-cadherin and enhanced activity of metalloproteinases. This leads to changes in the actin cytoskeleton indicating that DNAJA1

Figure 4. Effect of combination treatments on prostate cancer spheroids. (A) Cells were plated on Matrigel-coated 24 well plates. Six drugs (cabozantinib, clofarabine, vinblastine, idarubicin, omacetaxine and sorafenib) were tested on prostate cancer spheroids. These experiments were performed in triplicate and are average of 3 replicates from 3 different wells of a cell culture plate. The pictures are representative images as acquired using an EVOS cell imager. (B) Proliferation of spheroids treated with cabozantinib (CBZ), clofarabine (CFB), vinblastine (VBT), idarubicin (IRB), omacetaxine (OAT) and sorafenib (SRN) measured using AnnexinV/PI staining.
is important for prevention of the amoeboid-like transition of tumor cells. These studies indicated the involvement of DNAJA1 in maintaining cytoskeletal organization. We found 3 anticancer drugs targeting the cytoskeleton to be synergistic with DNAJA1 depletion, including vinblastine sulfate (cytoskeletal inhibitor that disrupts microtubule polymerization during mitosis and interferes with glutamic acid metabolism), estramustine (binds to microtubule-associated proteins (MAPs) and inhibits microtubule dynamics) and ixabepilone (promotes tubulin polymerization and microtubule stabilization, thereby arresting cells in the G2-M phase). Strikingly, two of the tubulin inhibitors were found to be antagonistic to DNAJA1 depletion. These include paclitaxel and ixabepilone. Paclitaxel inhibits the disassembly of microtubules resulting in the inhibition of cell division whereas Ixabepilone promotes tubulin polymerization and microtubule stabilization, arresting cells in the G2-M phase of the cell cycle. This apparent discrepancy may be explained by off-target effects of these molecules (see below).

Epigenetic modifying drugs display substantially modified potency depending on cellular DNAJA1 status. While previous studies have indicated the association between proteomic changes and histone PTMs in response to Hsp90 inhibitor treatment in bladder carcinoma cells, no such association has been shown for DNAJA1 and Histone PTMs. Interestingly, vorinostat was the only drug that was synergistic to DNAJA1 inhibition. It is a histone deacetylase inhibitor that binds to the catalytic domain of the histone deacetylases (HDACs). However, we also identified two histone deacetylase inhibitor drugs to be antagonistic to DNAJA1 depletion, panobinostat and romidepsin. These inhibit histone deacetylase (HDAC) which may impact cell cycle protein expression, cell cycle arrest in the G2/M phase and apoptosis. Excitingly, our data suggest a functional link between histones, their modifications and DNAJA1. While these findings require further investigation, it is possible that DNAJA1 may regulate the stability of histones themselves or histone chaperones. Interestingly, bortezomib (a proteasome inhibitor) lost potency when DNAJA1 was either inhibited with 116-9e or knocked out with CRISPR. Interestingly, a similar phenomenon has been observed in B16F10 melanoma cells. While treatment of these cells with 10 μM bortezomib was cytotoxic, this effect was abrogated with a combination of both quercetin (an Hsp70 inhibitor) and bortezomib. This apparent antagonism may be explained by their mechanism of action on the heat shock transcription factor, HSF1. While bortezomib acts to trigger the heat shock response in some cancers, the Hsp70/co-chaperone system maintains HSF1 in an active immature form. It is interesting to note that while there are clear classes of drugs that are made more potent by loss of DNAJA1 function (DNA damage response, cytoskeletal function etc.), there are a small number of drugs in these classes that are not impacted at all or even made less potent. This apparent discrepancy implies that some of these inhibitors might have multiple cellular targets in addition to their proposed primary mechanism of action. This theory has been validated in fascinating studies comparing making effects of small molecule therapies, gene knockout and knockdowns that theoretically target the same genes.

As in the case of any chemogenomic screen, care must be taken to validate screening results with other methods. While CRISPR KO cell lines offer the advantage of complete, specific and permanent gene silencing as compared to transient inhibition seen with small molecules or siRNA, the authors acknowledge that permanent knockout lines may respond by overexpressing/suppressing other genes to compensate, leading to non-specific effects. In this study, we took the approach of following up our initial screen with small molecule validation in 2D and 3D cell culture models. Going forward, we intend to validate several of these hits in vivo (mouse) model systems. 116-9e is a relatively new and unexplored molecule; while it clearly alters JDP binding, the exact mechanism of action on the heat shock transcription factor, HSF1 is not entirely clear. While bortezomib acts to trigger the heat shock response in some cancers, the Hsp70/co-chaperone system maintains HSF1 in an active immature form. It is interesting to note that while there are clear classes of drugs that are made more potent by loss of DNAJA1 function (DNA damage response, cytoskeletal function etc.), there are a small number of drugs in these classes that are not impacted at all or even made less potent. This apparent discrepancy implies that some of these inhibitors might have multiple cellular targets in addition to their proposed primary mechanism of action. This theory has been validated in fascinating studies comparing making effects of small molecule therapies, gene knockout and knockdowns that theoretically target the same genes.

Recent studies from our group and other have described the clear impact of Hsp70/JDP inhibition on individual oncoprotein client stability and prostate cancer cell survival. Overall, this study demonstrates the larger feasibility of inhibiting Hsp70 co-chaperones such as DNAJA1 as a novel anticancer therapy, acting to fine-tune Hsp70 function rather than completely abolishing it. Nearly a third of the anticancer compounds screened demonstrated increased potency in DNAJA1 knockout cells. Rather than attempting to develop co-chaperone inhibitors as a monotherapy, we believe their strength lies as sensitizing agents to existing therapies. Moreover, our data imply that overexpression of DNAJA1 in patient tumors may impact the effectiveness of a number of commonly used anticancer drugs. While further experiments characterizing the specific DNAJA1-mediated effects in cancer proliferation and the specificity of drugs such as 116-9e are required, our studies suggest perhaps a future precision medicine approach that uses tumor DNAJA1 status to guide treatment strategy.

Materials and methods

Cell culture. The HAP1 Chronic Myelogenous Leukemia cancer cell line and DNAJA1 knockout cell line was purchased from Horizon Discovery and were cultured in Iscove's Modified Eagle Medium (Invitrogen) with 10% fetal bovine serum (FBS, Clontech), 100 units/ml penicillin and 100 μg/ml streptomycin at 5% CO2 and 37°C. The LNCaP cancer cell line was purchased from ATCC and were cultured in RPMI-1640 medium (Invitrogen) with 10% fetal bovine serum (FBS, Clontech), 100 units/ml penicillin, and 100 μg/ml streptomycin at 5% CO2 and 37°C.

Drug screening. Approved Oncology Drug plates consisting of the most current FDA approved anticancer drugs were obtained from the National Cancer Institute (NCI). For experiments delineating the synergy between...
the loss of DNAJA1 and approved anticancer drug, HAP1 cells and HAP1 (DNAJA1 KO) cells were plated in growth media at 20% confluency 1 day prior to drug treatment. On Day 1 of treatment, cells were treated with DMSO (control), Approved oncology anticancer drugs at 50 µM for 72 h. Following drug treatments, Cell Titer-Glo reagent was added directly to the wells according to the manufacturer's instructions. The luminescence was measured on Bio-Tek Plate reader. Luminescence reading was normalized to and expressed as a relative percentage of the plate averaged DMSO control. The data shown are the mean and SEM of three independent biological replicates.

**Combination index (CI) calculations.** For IC<sub>50</sub> calculations, LNCaP cells were seeded in triplicates in 96-well white bottom Nunc plates in growth media at 20% confluency 1 day prior to initiation of drug treatment. On Day 1 of treatment, cells were treated with DMSO (control) and ten folds serial dilution of anti-cancer drugs cabozantinib, clofarabine, vinblastine, sorafenib, idarubicin and omacetaxine mespucinicate and 116-9e. After 72 h, cell viability was measured using Promega Cell Titer-Glo cell viability assay on Bio-Tek plate reader. The combination index was calculated using the Chou-Talalay method using CompuSyn software.

**Spheroid generation.** Single-cell suspensions (5,000/well) were plated in one well of 24-well plates in a 1:1 mixture of RPMI medium and Matrigel (BD Bioscience CB-40324). Cells in Matrigel were kept cold at all times and under continuous agitation. Warm PBS was added to all empty wells, if any. Plates were incubated at 37 °C with 5% CO<sub>2</sub> for 15 min to solidify the gel before addition of 100 µl of pre-warmed RPMI to each well. Two days after seeding, the media was fully aspirated and replaced with fresh RPMI containing the indicated drugs. The same procedure was repeated daily on two consecutive days. Twenty-four hours after the last treatments, the media was aspirated and the wells were washed with 100 µl of pre-warmed PBS. To prepare for downstream assays, spheroids were released from the Matrigel by incubating at 37 °C for 40 min in 100 µl of 10 mg/mL Dispase (Sigma).

**Apoptosis assay.** Apoptosis of LNCaP spheroids was detected by the Annexin V–FITC/propidium iodide-binding assay. Cells were treated with either 0.1% DMSO (dimethyl sulfoxide),116-9e, cabozantinib, clofarabine, vinblastine, sorafenib, idarubicin, omacetaxine mespucinicate and sorafenib alone or in combination with 116-9e for 48 h at the IC<sub>50</sub> concentrations, and then stained with Annexin V–FITC and propidium iodide. The rate of apoptosis was determined using a BD LSR Fortessa flow cytometer, and the collected data were analyzed using FlowJo software. Apoptosis was reported as the mean ± SD. The results are representative of three independent experiments.

**Bioinformatics.** Cancer genome data and Cancer Cell Line Encyclopedia data were accessed from the cBioPortal (www.cbioportal.org) for Cancer Genomics. Total patient numbers and detailed information regarding published datasets and associated publications are indicated in Fig. 1A,B.

**Statistical analysis.** Data were analyzed using GraphPad Prism built-in statistical tests indicated in relevant figure legends. The following asterisk system for P-value was used: P < 0.05; P < 0.01; 0.001; and P < 0.0001.

**Western blotting.** Protein extracts were made as described. 30 µg of protein was separated by 4–12% NuPAGE SDS-PAGE (Thermo). Proteins were detected using the following antibodies; anti-DNAJA1/HDJ2 (Thermo # MAS-12748), anti-Actin (CST # 9774), Anti-Hsc70 (Santa Cruz, # sc-7298), anti-Hsp70 (Enzo # C92F3A 5), anti-Hsp90 (Thermo # MA5-12748), anti-Actin (CST # 9774), Anti-Hsc70 (Santa Cruz, # sc-7298), anti-Hsp70 (Enzo # C92F3A 5), anti-Hsp90 a/b (Santa Cruz # sc-13119), anti-Bag3 (Santa Cruz # sc-136467), anti-Hsp110 (Stress Marq, # SPC-195,) at 1:4,000 dilution in TBST + 1% BSA. Blots were imaged on a Chemi Doc MP imaging system (Bio-Rad).

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**References**

1. Rosenzweig, R., Nillegoda, N. B., Mayer, M. P. & Bukau, B. The Hsp70 chaperone network. Nat. Rev. Mol. Cell Biol. 20, 665–680. https://doi.org/10.1038/s41580-019-0133-3 (2019).
2. Ciocca, D. R. & Calderwood, S. K. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 10, 86–103. https://doi.org/10.10379/csc-99r1 (2005).
3. Gestwicki, J. E. & Shao, H. Inhibitors and chemical probes for molecular chaperone networks. J. Biol. Chem. 294, 2151–2161. https://doi.org/10.1074/jbc.T110.1185213 (2019).
4. Evans, C. G., Chang, L. & Gestwicki, J. E. Heat shock protein 70 (hsp70) as an emerging drug target. J. Med. Chem. 53, 4585–4602. https://doi.org/10.1021/jm100054f (2010).
5. Kampina, H. H. & Craig, E. A. The Hsp70 chaperone machinery: I proteins as drivers of functional specificity. Nat. Rev. Mol. Cell Biol. 11, 579–592. https://doi.org/10.1038/nrm2941 (2010).
6. Craig, E. A. & Marszalek, J. How do I-Proteins get Hsp70 to do so many different things?. Trends Biochem. Sci. 42, 355–368. https://doi.org/10.1016/j.tibs.2017.02.007 (2017).
7. Nitika & Truman, A. W. Cracking the Chaperone Code: cellular roles for Hsp70 phosphorylation. Trends. Biochem. Sci. 42, 932–935. https://doi.org/10.1016/j.tibs.2017.10.002 (2017).
48. Cerami, E. et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.*, 2, 401–404. https://doi.org/10.1158/2159-8290.CD-12-0085 (2012).

49. Nitika et al. Chemogenomic screening Identifies the Hsp70 Co-chaperone HDJ2 as a Hub for Anticancer Drug Resistance. *bioRxiv*, 818427, doi:https://doi.org/10.1101/818427 (2019).

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**Author contributions**

N. performed all main experiments and wrote manuscript drafts; J.S.B. assisted with the chemogenomic screen; J.E.T. and L.E.K. helped with data analysis, figure drawing and manuscript editing; S.K.C. and A.W.T. conceived the experiments and edited the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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