Advances in Experimental Medicine and Biology 1243

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HSF1 and Molecular Chaperones in Biology and Cancer

Springer

Advances in Experimental Medicine and Biology

Volume 1243

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2018 Impact Factor: 2.126.

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-40203-7 ISBN 978-3-030-40204-4 (eBook)
https://doi.org/10.1007/978-3-030-40204-4

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface: Dedication to Susan Lindquist

Proteostasis, or protein homeostasis, is a fundamental requirement for fitness in cells across all kingdoms of life. The proteostasis network encompasses pathways that synthesize, fold, and degrade cellular proteins. Deregulation of these pathways has devastating consequences to organismal health, leading to a range of pathological conditions including developmental defects, neurodegenerative diseases, and cancer. The compendium of chapters in this book focuses on the structure, function, and therapeutic implications of the proteostasis machinery in cancer.

We have been fortunate to receive contributions from an extraordinary group of basic, clinician, and translational scientists with a range of research approaches and expertise that include biochemistry, molecular biology, genetics, structural biology, and chemical biology. Underscoring the deep conservation and critical importance of this field of study, these researchers employ model systems from yeast to human to interrogate the underlying mechanisms and functional significance of the proteostasis network in health and disease. While a diverse group, these scientists are unified by a passion and belief that understanding the mechanistic basis by which proteins – the molecular machines of the cell – are able to fold and function properly is critical to understanding nearly all aspects of tumorigenesis. The chapters in this book reflect this fundamental tenet and cover the structural and biochemical properties of the major chaperone systems in the cell, how these chaperone systems function to impact cancer cells and the tumor microenvironment, and the promise of targeting these systems as an anticancer therapeutic strategy.

We chose to take this initiative to celebrate and commemorate the work of our beloved late mentor, Susan Lee Lindquist, a pioneer of proteostasis. Susan started her work on heat-shock proteins in the 1970s as a PhD student in the lab of Matthew Meselson at Harvard University. After completion of her PhD in 1976 and a brief postdoctoral fellowship at the University of Chicago, she joined the faculty at the University of Chicago where she embarked her independent research on protein synthesis and folding before moving to the Whitehead Institute for Biomedical Research at MIT. Switching to new model organisms as the biological questions required, Susan electrified the field time and time again using cutting-edge methods and incisive experiments to reveal new concepts that laid the foundation of what would later become known as proteostasis.

Her work on heat-shock proteins, prions, and amyloids in model organisms led her to realize that the interface between protein homeostasis and environmental stress might be involved in the evolution of new traits. This insight crystalized into the paradigm-shifting concept of Hsp90 as a capacitor of evolution. A decade later, she showed that similar protein-based evolutionary mechanisms fuel the malignant progression and therapeutic resistance of cancers.

Sadly, Susan succumbed to the disease she had been studying. On 27 October 2016, she died of ovarian cancer at the age of 67. The scientific community lost a hero that day. A scientific pioneer, role model, and advocate for women in STEM, Susan's impact lies not only in her discoveries but also in her contributions to the culture of science. Her mentorship, the collegial environment she fostered, her curiosity, and her scientific enthusiasm made her into the iconic scientist she was and whom we dearly miss.

This book is dedicated to you, Sue.

Chicago, IL, USA — Marc Laurence Mendillo
Chicago, IL, USA — David Pincus
Rehovot, Israel — Ruth Scherz-Shouval

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1 Structural and Biochemical Properties of Hsp40/Hsp70 Chaperone System

Ofrah Faust and Rina Rosenzweig

Abstract

Hsp70s are ubiquitous molecular chaperones that act in a myriad of cellular functions, affecting virtually all aspects in the life of proteins from synthesis to degradation. Hsp70 proteins act in the cell in cooperation with a large set of dedicated co-chaperones consisting of J-domain proteins and nucleotide exchange factors that regulate the Hsp70 chaperone cycle. Recent studies have made significant progress towards obtaining a better understanding of the mechanisms through which Hsp70s and their co-chaperones operate, providing insights into structural, kinetic, and functional features of the various members of this network. In this chapter we describe the emerging working principles of the Hsp70 machine and its co-chaperones, and highlight how mechanistic aspects of this network are tied to distinct protein folding functions.

Keywords

Hsp70 chaperones · J-domain proteins · Nucleotide exchange factors · Protein folding · Heat shock proteins · Quality control · DnaK

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1.1 Hsp70 Chaperone System

In order to survive, organisms must be able to maintain cellular homeostasis in a constantly changing environment. Molecular chaperones are essential to this effort, as they provide a "buffer" that helps protect cellular proteins from the damaging effects of extreme conditions, such as sudden increase in temperature, oxidative stress, exposure to heavy metals, hypoxia, and metabolic dysfunction (Balchin et al. 2016; Craig 2018; Fernandez-Fernandez et al. 2017; Nillegoda et al. 2018; Mogk et al. 2018).

In fact, molecular chaperones were initially defined as heat shock proteins (HSPs), as their protein levels in almost all organisms were highly increased in response to elevated temperatures (Lindquist 1986). As more has been discovered regarding molecular chaperones, however, this group has more broadly been defined as consisting of any protein that assists the correct non-covalent assembly of other polypeptide-containing structures *in vivo*, but which is not a component of these assembled structures when they perform their normal biological functions.

Hsp70 proteins are a textbook case of this behavior and belong to a ubiquitous and abundant family of molecular chaperones that regulates protein quality control and homeostasis in a stunningly wide array of cellular processes (Balchin et al. 2016; Craig 2018; Fernandez-Fernandez et al. 2017; Nillegoda et al. 2018; Mogk et al.

M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_1

2018). Members of this family play key roles in functions such as folding of newly synthesized proteins, stabilization and refolding of misfolded proteins, solubilization of protein aggregates, protein trafficking, and proteolytic degradation of unstable proteins. The many housekeeping and stress-associated protein-folding activities in which Hsp70s participate not only underscore the critical importance of these chaperones for the maintenance of protein homeostasis, but also link them to numerous pathophysiological conditions in humans (Qu et al. 2015), including neurodegenerative diseases, cancer, and organismal aging (Walther et al. 2015).

Not surprisingly, then, Hsp70s are amongst the most abundant chaperones in the cell, accounting for as much as 0.5–2% of the total cellular protein mass (Finka et al. 2015). In humans, there are at least 13 distinct genes, located on several different chromosomes, that encode for Hsp70 isoforms (Kampinga and Craig 2010), with several of these being distinct from the canonical, cytosolic Hsp70s by differences not only in their localization (Table 1.1), but also

Table 1.1 The Hsp70 family of molecular chaperones

Hsp70 chaperones		Cellular localization	Properties	Alternative names
Human	HSPA1A	Cytosol, nucleus, and nucleoli upon heat shock	Strongly stress inducible	HSP70-1, Hsp72
	HSPA1B	Cytosol, nucleus, and nucleoli upon heat shock	Strongly stress inducible	HSP70-2, Hsp70-2
	HSPA1L	Mostly cytosol under basal conditions. Nucleus but not nucleoli upon heat shock	Constitutively expressed	HSP70-hom, Hsp70-1L, Hsp70-1t, Hum70t
	HSPA2	Nucleus	Constitutively expressed	Hsp70.2
	HSPA5	Endoplasmic reticulum (ER)	Constitutively expressed	Grp78, BiP, Mif-2
	HSPA6	Cytosol and nucleus	Stress inducible, no basal expression	HSP70B’
	HSPA8	Cytosol, nucleus, and cell membrane	Constitutively expressed. Moderately induced by stress	Hsc70, Hsc71, Hsp71, Hsp73
	HSPA9	Mitochondria	Constitutively expressed	Grp75, HspA9B, MOT, MOT2, mtHsp70, mortalin
	HSPA12A	Intracellular	Constitutively expressed	
	HSPA12B	Intracellular	Constitutively expressed	
	HSPA14	Cytosol	Ribosomes associated Hsp70 Stress inducible	Hsp70L1
Yeast	Ssa1	Cytosol and nucleus	Constitutively expressed	
	Ssa2	Cytosol and nucleus	Constitutively expressed	
	Ssa3	Cytosol and nucleus	Stress inducible	
	Ssa4	Cytosol and nucleus	Stress inducible	
	Ssb1	Cytosol and nucleus	Ribosome targeting Hsp70	
	Ssb2	Cytosol and nucleus	Ribosome targeting Hsp70	
	Kar2	Endoplasmic reticulum (ER)	Induced by ER stressors	Grp78, Bip
	Ssc1	Mitochondria	Constitutively expressed Involved in pre-protein import into the mitochondrial matrix	mtHSP70, Ens1
	Ssq1	Mitochondria	Involved in iron-sulfur cluster biogenesis	Ssh1, Ssc2
	Ssc3	Mitochondria		Ecm10
	Ssz1	Cytosol	Ribosomes associated Hsp70	Pdr13
Bacteria	DnaK	Cytosol	Stress inducible	
	HscA	Cytosol	Involved in iron-sulfur cluster biogenesis	
	HscC	Cytosol		ybeW

in their substrate recognition and allosteric control. Levels of Hsp70s are tightly regulated according to cellular needs (e.g. growth or tissue-specific functions), and Hsp70 family members exist in most cell compartments (cytoplasm, nucleus, ER, mitochondria, chloroplasts). Some members even associate directly with target sites such as ribosomal tunnel exits and membrane translocons where nascent and translocating substrates emerge (Craig 2018; Zhang et al. 2017). Hsp70 chaperones do not perform their tasks alone, though, but rather rely on cooperation with an extensive network of co-chaperones from the J-domain protein family (DnaJs; Hsp40s (Kampinga and Craig 2010)), as well as nucleotide exchange factors (NEF) that regulate their activity (Abrams et al. 2014; Bracher and Verghese 2015). Hsp70s also cooperate with other protein quality control systems, including small heat shock proteins; refolding chaperones and chaperonins, such as Hsp90 and GroEL/TriC; and Hsp100 disaggregation machineries, allowing further functional diversification (Balchin et al. 2016; Rosenzweig et al. 2019).

1.1.1 Hsp70 Domain Structure and Functional Cycle

Structurally, Hsp70s consist of an N-terminal nucleotide-binding domain (NBD) with ATPase activity, and a substrate-binding domain (SBD) that can be further divided into a 15 kDa polypeptide binding cleft (SBDβ), and a 10 kDa α-helical lid (SBDα) (Mayer and Bukau 2005) (Fig. 1.1a). The NBD has an actin-like fold consisting of four subdomains (IA, IB, IIA, IIB) arranged in two lobes separated by a deep cleft (Fig. 1.1b), with ATP binding being coordinated by all four subdomains (Flaherty et al. 1990). SBDβ is composed of an eight-stranded β-sandwich containing the substrate binding cavity and its central hydrophobic pocket (Morshauser et al. 1995; Zhu et al. 1996; Kityk et al. 2015), which typically interacts with short stretches enriched in aliphatic side chains (Morshauser et al. 1995; Zhu et al. 1996; Mayer and Kityk 2015) (Fig. 1.1c). NBD and SBD are connected by a highly conserved flexible linker that is essential for the allosteric mechanism coupling nucleotide and polypeptide binding (Vogel et al. 2006; Alderson et al. 2014; Zhuravleva and Gierasch 2011).

The nucleotide binding state of Hsp70 dictates the chaperone's substrate-binding affinities. In the ATP-bound state, the Hsp70 NBD and SBD are docked to each other, and the helical lid is in an open state, allowing the rapid binding and release of substrates from the binding cleft (Fig. 1.1d). Upon ATP hydrolysis, however, this docked conformation of Hsp70 changes dramatically – the SBD dissociates from the NBD and the α-helical lid completely or partially covers the polypeptide-binding cavity (Marcinowski et al. 2011; Schlecht et al. 2011), preventing substrate dissociation and resulting in an up to 100 fold increase in affinity for substrates.

ATP hydrolysis therefore acts as a switch between two conformational states and is key to the Hsp70 chaperone cycle. Because the intrinsic ATPase activity of Hsp70 is very low (approximately 1 ATP molecule per 6–40 min), Hsp70s do not generally act alone, and instead rely on co-chaperones from the J-protein family (J-domain proteins; Hsp40s) to stimulate their ATPase activity (Kampinga and Craig 2010) (Fig. 1.1e). Surprisingly, while J-proteins are strictly required for Hsp70 function, they themselves only modestly stimulate Hsp70 ATPase activity (Kityk et al. 2018). Similarly, interaction with substrates was also observed to only elicit a slight increase in Hsp70 ATP hydrolysis (Kityk et al. 2018). Synergistic binding to both substrates and J-proteins, however, stimulates Hsp70 ATP hydrolysis rates several thousand-fold, converting Hsp70 to the high affinity ADP state and providing an efficient mechanism for trapping client polypeptides (Kityk et al. 2018; Mayer 2013) (Fig. 1.1e).

As most Hsp70s also bind the resulting ADP with high affinity, a nucleotide exchange factor (NEF) is required to stimulate ADP release, thereby allowing ATP to rebind. Then, upon this ATP binding, Hsp70 will undergo conformational changes that facilitate the release of the substrate, thereby allowing a new interaction cycle to begin (Mayer 2013) (Fig. 1.1e). Hsp70s

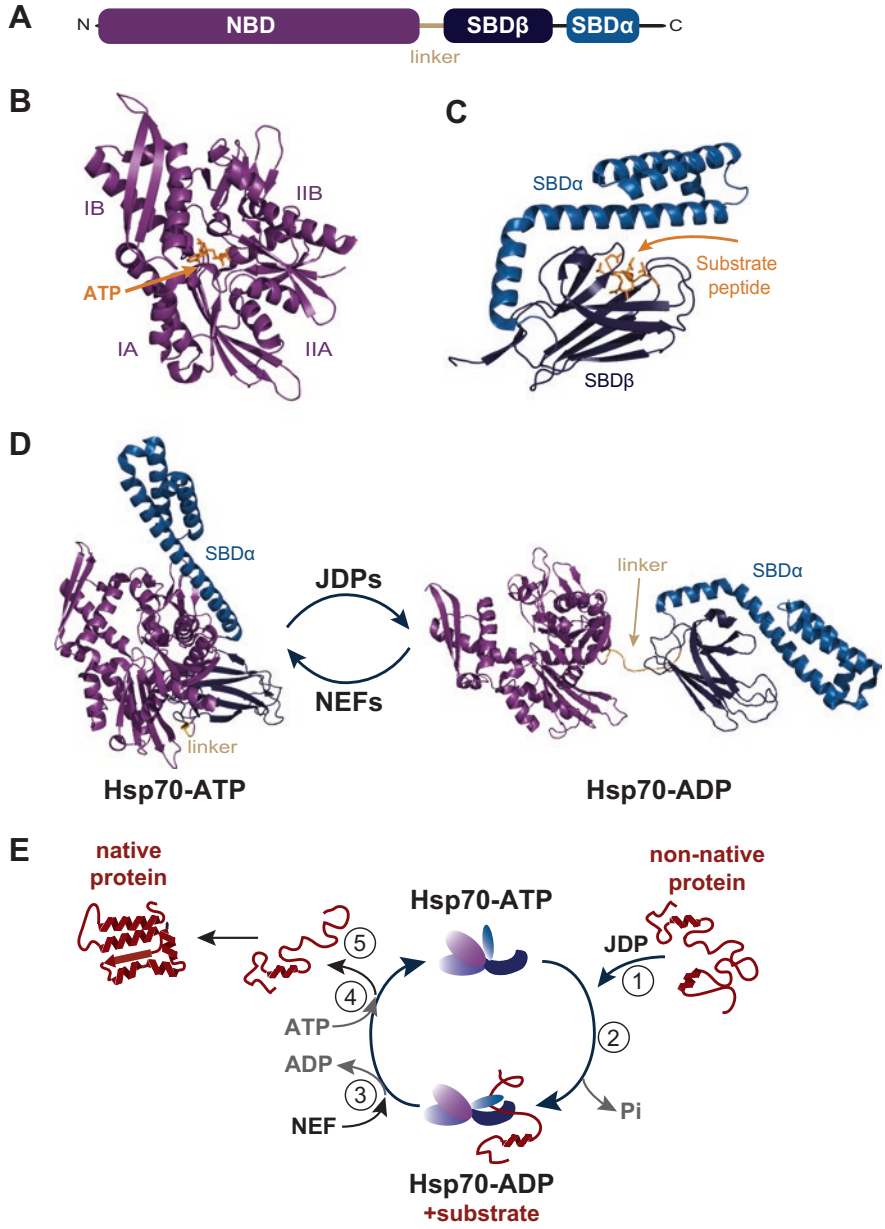

Fig. 1.1 Functional cycle and structure of Hsp70 chaperones
(**a**) Schematic representation of the domain structure of Hsp70. (NBD) is shown in purple; SBDβ, dark blue; SBDα, light blue; and the flexible linker connecting the NBD to SBD is colored light orange. (**b**) Cartoon representation of Hsp70 structure in the ATP bound state with subdomains IA, IIA, IB, and IIB denoted (PDB ID 4B9Q (Kityk et al. 2012)). (**c**) Structure of Hsp70 substrate binding domain in the high-substrate-affinity state, in complex with a peptide (PDB ID 1DKX (Zhu et al. 1996)); SBDβ is colored dark blue; SBDα, light blue; peptide, orange). (**d**) The conformational changes of Hsp70 upon ATP hydrolysis. Left – Hsp70 in the ATP state (PDB ID 4B9Q (Kityk et al. 2012)), Right – Hsp70 in the ADP-bound state (PDB ID 1KHO (Bertelsen et al. 2009)). Domains are colored as in B. (**e**) Schematic of the allosteric mechanism of Hsp70 molecular chaperones, showing steps that are facilitated by J domain and nucleotide exchange factor (NEF) co-chaperones. The Hsp70 functional cycle involves the following steps: (1) JDP-mediated delivery of substrate to ATP-bound Hsp70 (2) JDP-mediated hydrolysis of ATP to ADP, resulting in conformational changes of the Hsp70 and transition to the high-substrate-affinity ADP state (3). NEF-induced ADP dissociation (4) binding of ATP, which converts the Hsp70 to the low-substrate affinity state, leading to substrate release (5). Released substrate either folds to native state or, alternatively, re-enters the Hsp70 cycle

are thought to undergo multiple such consecutive bind and release cycles with their client proteins, in which the kinetics are custom-tailored to the needs of the particular client protein through interaction with co-chaperones (Clerico et al. 2015; Mashaghi et al. 2016).

1.1.2 Substrate Recognition and Remodeling by the Hsp70 Chaperones

Hsp70 chaperones are able to interact with an amazingly wide range of client types, including nascent (unfolded) polypeptides emerging from the ribosome, folding intermediates, natively folded proteins (e.g. the heat shock transcription factor, clathrin-coated vesicles, and replication initiation protein), misfolded proteins, and even protein aggregates and amyloid fibers (Rosenzweig et al. 2019; Clerico et al. 2019). How these chaperones recognize such a diversity of protein conformations has therefore been a matter of great interest.

Part of the answer comes from the early peptide library studies of the Hsp70 bacterial homolog, DnaK. These showed that the chaperone has a preference for 5-residue hydrophobic cores enriched in aliphatic amino acids, and flanked by positively charged regions on both sides (Rudiger et al. 1997). Such Hsp70-binding motifs are found in most proteins, but are usually buried in the hydrophobic core of the proteins when they are in their native folded state. These motifs are exposed, however, during protein synthesis, or as a result of heat stress or oxidative damage that cause protein denaturation and misfolding.

The first structural description of an Hsp70-substrate complex revealed that the model peptide (NRLLLTG) is bound to the DnaK SBD in an extended conformation (Zhu et al. 1996). This occurred along a hydrophobic cleft in the SBD, with the side-chain of the peptide's central leucine residue projecting into a small hydrophobic binding pocket (Fig. 1.1c). In addition, over a stretch of five consecutive residues the peptide backbone was further enclosed by the SBDβ cleft and stabilized through a network of hydrogen bonds. While this peptide-binding configuration is evolutionarily conserved (Morshauser et al. 1999; Pellecchia et al. 2000; Stevens et al. 2003; Cupp-Vickery et al. 2004; Jiang et al. 2005), variability has been observed with respect to the register and orientation of peptides bound to Hsp70s (Clerico et al. 2015; Zahn et al. 2013; Tapley et al. 2005), indicating that substrate binding exhibits some degree of plasticity and promiscuity.

In addition to promiscuity in substrate recognition, further heterogeneity may originate from differences in the Hsp70s themselves. While the structure of the Hsp70 SBD is largely conserved (Morshauser et al. 1999; Pellecchia et al. 2000; Stevens et al. 2003; Cupp-Vickery et al. 2004; Jiang et al. 2005), it becomes more and more evident that Hsp70s originating from different organisms, and even Hsp70s from the same organisms but from different compartments, differ in their substrate recognition preferences (Fourie et al. 1994) (see Table 1.1 for the list of Hsp70 chaperones in different organisms). For example, while cytosolic Hsp70s preferably bind leucine-enriched peptide stretches, the ER homolog, BiP, rather engages motifs containing aromatic residues (Gragerov and Gottesman 1994). A striking difference was also reported in the substrate specificity of the *E. coli* HscA and the *S. cerevisiae* mitochondrial Hsp70 Ssq1, which are involved in the biogenesis of Fe-S cluster proteins. Both chaperones, instead of leucines, preferentially recognize peptide sequences enriched in prolines (Dutkiewicz et al. 2004; Hoff et al. 2002). HscC, also in *E. coli*, further differs in substrate specificity from both HscA, and their third counterpart, DnaK (Kluck et al. 2002), and similarly, differences in substrate specificity were reported for human Hsp70 (HSPA1A) and Hsc70 (HSPA8) (Taipale et al. 2014; Mok et al. 2018). Beyond binding preferences, the kinetics of substrate interaction also vary greatly between different Hsp70s, with the eukaryotic Hsp70 chaperones displaying faster binding and release rates compared to their prokaryotic homologs (Marcinowski et al. 2013). In all, variations in binding specificities and kinetics are not only widespread between the members of the Hsp70

chaperone family, but are most likely drivers, at least in part, of Hsp70 functional diversity.

Perhaps one of the best established Hsp70 functions is interaction with nascent proteins as they exit the ribosome. There, the proposed role of Hsp70s is to delay folding of an emerging protein domain until all the sequence elements required for folding are accessible, thereby preventing the formation of non-native interactions and protecting the nascent chain from misfolding and aggregation (Balchin et al. 2016; Preissler and Deuerling 2012; Frydman 2001; Kramer et al. 2009). Some Hsp70s perform these holding functions by transient association with the ribosome, employing specialized targeting machinery, with the archetype being the yeast Hsp70, Ssb (Ssb1 and Ssb2). This chaperone is targeted to the exit of the ribosomal tunnel by the ribosome-associated complex (RAC), which is composed of the J-like protein, zuotin and the non-canonical yeast Hsp70, Ssz1 (Zhang et al. 2017; Lee et al. 2016). Ssb interacts with a large spectrum of emerging polypeptides (Willmund et al. 2013; Doring et al. 2017) by transient association with exposed hydrophobic stretches typically found in the core of folded protein domains (Doring et al. 2017). Nascent proteins can bind Ssb or even the canonical yeast Hsp70s, Ssa1–4, repeatedly during chain elongation, preventing misfolding of the mature protein (Meyer et al. 2007).

An additional example of Hsp70 interaction with unfolded proteins is during translocation of newly synthesized proteins into mitochondria, chloroplasts and the ER membranes (Craig 2018). Hsp70 binding to cytosolic precursors of these proteins keeps them in an unfolded state until a targeting machinery engages the precursors for delivery to the translocon (Craig 2018). At the *trans* side of the membranes, these polypeptide chains are then engaged by compartment specific, translocon-associated Hsp70s that help to pull the unfolded protein into the organelle and facilitate folding. Interestingly, the necessary pulling forces for this transmembranal translocation are not a product of chemical hydrolysis, but rather as a result of the low intrinsic entropy state created by the limited conformational space of the Hsp70 molecules bound next to the translocation pore. Inward movement of the translocating polypeptide, in turn, increases the available conformational space, thereby increasing entropy and generating an entropic pulling force (Craig 2018; Goloubinoff and De Los Rios 2007). Thus, through mere localized binding, Hsp70 can produce enough force (10–20pN) (Goloubinoff and De Los Rios 2007) to drive protein translocation.

While many Hsp70 clients are unfolded proteins, the chaperone is also known to interact with non-native/misfolded and, in some cases, even native, fully-folded proteins, provided their binding sites are accessible to the Hsp70 SBD. What happens to these clients, however, once they are in complex with Hsp70?

One of the earliest studies, using circular dichroism and fluorescence, of unstable proteins bound to the bacterial Hsp70 homolog, DnaK, indicated that bound substrate proteins are significantly unfolded (Palleros et al. 1994). This observation was further supported by single-molecule fluorescence spectroscopy studies of rhodanese in complex with DnaK, detecting a largely expanded unfolded conformation of the client protein while bound to the chaperone (Kellner et al. 2014).

More recently, NMR spectroscopy studies of Hsp70 with various, single-domain clients demonstrated that clients associated with Hsp70 exist in a conformationally heterogeneous, but primarily unfolded ensemble (Lee et al. 2015; Sekhar et al. 2015, 2016; Rosenzweig et al. 2017). Interestingly, in these clients, certain local structural propensities of the folded state were maintained both when the proteins were free in solution, and when they were bound to the Hsp70 chaperone (Sekhar et al. 2015). Further investigation suggested that DnaK specifically disrupted tertiary, long range contacts, while enabling local structure formation (Sekhar et al. 2016). Subsequent studies further showed that Hsp70s selectively bind to client conformations that, even transiently, expose hydrophobic binding motifs, thereby reshaping the folding energy landscape of the client and increasing the probability of achieving their proper fold (Sekhar et al. 2018).

As Hsp70s generally bind regions in their clients that are either stably or transiently exposed (Sekhar et al. 2018), one possibility is that when native clients are bound by Hsp70s, it is in linker or loop regions, while the rest of the protein remains folded. Such a mode of binding was described for a natural Hsp70 substrate, a well-folded clathrin triskelion (a trimer of clathrin heavy- and light-chain dimer), with Hsp70 binding to the QLMLT motif present in the clathrin C-terminal unstructured tails (Bocking et al. 2011). There, Hsp70 conformationally selects clathrin states that are incompatible with the assembled triskelions, thereby disassembling the clathrin cages and uncoating the budding vesicles during endocytosis (Fotin et al. 2004; Rapoport et al. 2008). Another example is DnaK, which binds to the unstructured loop region of E. coli heat shock transcription factor σ32 (Chakraborty et al. 2014; Rodriguez et al. 2008), that presumably renders the transcription factor amenable for degradation. The situation in both these cases is somewhat similar to the *in vitro* observed Hsp70 dimerization, where the SBD of one Hsp70 binds the flexible hydrophobic linker of the other, while leaving that chaperone intact (Chang et al. 2008).

Moreover, certain protein substrates may retain their tertiary structure even when Hsp70s bind to structured regions. Using disulfide cross-linking and measurement of mobility of spin labels, it was shown that the α-helical lid of the SBD does not necessarily fully close around protein substrates as it would around a peptide, potentially allowing for binding of more structured proteins (Schlecht et al. 2011). Similarly, single molecule FRET measurements between the lid and the SBD showed that while the lid is closed upon Hsp70 binding to a short, 10-residue peptide, complex formation with molten globule-like cellular substrate proteins results in a far lesser extent of lid closure (Banerjee et al. 2016).

While the function of Hsp70 binding to native proteins is still under investigation, it is clear that for unfolded and misfolded clients, the Hsp70-induced disruption of intramolecular contacts serves as an efficient way of rescuing proteins from kinetic traps that would otherwise lead to further misfolding and/or aggregation. Moreover, the resulting extended Hsp70-bound proteins may then pose a beneficial starting point for either spontaneous folding or presentation to downstream chaperone machineries.

1.2 Regulation of Hsp70 Function by Co-chaperones

Much of our mechanistic understanding of Hsp70 function comes from studies using the E. coli orthologs, which include a single Hsp70 (DnaK), a J-domain protein (DnaJ), and an NEF (GrpE). While the main players of the eukaryotic system are conserved, the diversity of the system has been greatly expanded over the course of evolution. As a result, the human genome currently contains more than 13 Hsp70s, 13 NEFs, and close to 50 J-domain proteins (JDPs) (Kampinga and Craig 2010), and this increase in potential partners has generated an enormous number of possible combinations. One reason for this expansion is localization of chaperones in specialized compartments, such as the cytoplasm, nucleus, ER, or mitochondria. Another potential explanation, however, could be the necessity for functional diversity, with this potentially being made possible via different combinations of Hsp70 (each with their own substrate specificities, levels of expression, and post-translational modifications), JDPs, and NEFs.

For instance, through specialized members of the JDP family, Hsp70s can associate with target sites such as ribosomal tunnel exits and membrane translocons, where nascent and translocating substrates emerge. Another example is the mammalian J-protein, auxilin, that is exclusively dedicated to helping Hsp70 dissociate clathrin triskelions. As other JDPs are unable to compensate for loss of auxilin, this suggests that auxilin, and potentially other co-chaperones, evolved to recruit Hsp70s into very specific functions.

The JDP-Hsp70 system, however, does not just act in isolation and often collaborates with other chaperone systems – thereby adding to its many possible roles. In folding or refolding pathways, for example, Hsp70s can hand off substantially unfolded proteins to Hsp90 or Hsp60

chaperonins for final stages of folding or activation. In other cases, Hsp70 systems have even been reported to pass client proteins to other Hsp70 systems in different compartments, thereby helping newly synthesized polypeptides reach their final cellular destination.

To help better understand this complex system of chaperones and their many possible interactions, in the following sections we will describe the key co-chaperones of the core Hsp70 machinery, and its interface with other, downstream chaperone systems.

1.2.1 J-Domain Proteins

J-domain proteins (JDPs, also known as DnaJs or Hsp40 proteins) are essential components of the Hsp70 chaperone system, as they both regulate Hsp70 chaperones by stimulating ATP hydrolysis and play an important role in initial substrate recognition and remodeling (Tiwari et al. 2013). JDPs are conserved in evolution and are defined by the presence of a J-domain, an approximately 70 residue highly conserved region containing four α-helices (Fig. 1.2a). The linker region between helices 2 and 3 is especially well conserved and contains the histidine-proline-aspartic acid (HPD) motif vital for the stimulation of Hsp70 ATP hydrolysis (Tsai and Douglas 1996) (Fig. 1.2a). J-domain proteins have historically been divided into three classes, based on the similarity of their domain arrangement to that of the bacterial DnaJ. Class A J-domain proteins (e.g. bacterial DnaJ, yeast Ydj1, and human DnaJA1–4) share four domains with the bacterial DnaJ: the N-terminal J-domain; a glycine-phenylalanine-rich linker segment of unclear function; two β-sandwich C-terminal domains, CTDI and II, that contain the substrate binding sites; and a zinc-finger like region (ZFLR) incorporated into CTDI (Fig. 1.2b, c). Class B J-domain proteins (e.g. the yeast Sis1, and human DnaJB1,4,5) share the J-domain, GF-rich linker and a C-terminal substrate binding domain (Cheetham and Caplan 1998). Class C J-domain proteins are the most heterogeneous group and share only the J-domain with DnaJ, with additional domains in these JDPs mainly serving to localize Hsp70s to specific organelles or transfer their substrates to downstream chaperones (Kampinga and Craig 2010) (Fig. 1.2b).

The three JDP classes also differ in their client specificities (Kampinga and Craig 2010), with class A and B JDPs, despite many similarities in their mode of substrate binding, recognizing distinct features in amorphous protein aggregates. Human DnaJA2, for example, preferentially binds to and assists solubilization of small aggregates (ca. 200–700 kDa), whereas DnaJB1 prefers larger species (ca. 700–5000 kDa) (Nillegoda et al. 2015, 2017). In contrast, class C J-domain proteins are generally considered to specifically interact with only a limited number of protein substrates or not to interact directly with proteins at all (Kampinga and Craig 2010). Even within the same family, the binding kinetics of different J-domain proteins with their substrates can vary greatly, from very transient to rather stable, with some JDPs having holding capabilities in their own right (e.g. DnaJB6 and DnaJB8, which are capable of efficiently blocking amyloid formation and the aggregation of misfolded proteins) (Szabo et al. 1996; Linke et al. 2003; Hageman et al. 2010; Kakkar et al. 2016). In all, there are approximately 50 different members of the J-domain protein family encoded in the human genome, ranging in size from 10 to 520 kDa (Kampinga and Craig 2010). This variety in size, stemming from a multitude of different domain structures, is also reflected in the functional diversity of the different JDPs.

Functionally, interaction of purified J-domain with Hsp70 chaperones has been shown to be sufficient for the enhancement of Hsp70 ATPase activity, and crucial missing insight into the molecular mechanism of J-domain action was recently provided by the solved X-ray structure of the *E. coli* DnaJ J-domain in complex with the ATP-bound open conformation of DnaK (Kityk et al. 2018). There, the J-domain binds at the interface between NBD and SBDβ, on top of the interdomain linker, forming polar contacts with these two domains and hydrophobic contacts with SBDβ and the linker (Fig. 1.2d). This mode of binding also explains the selective interaction

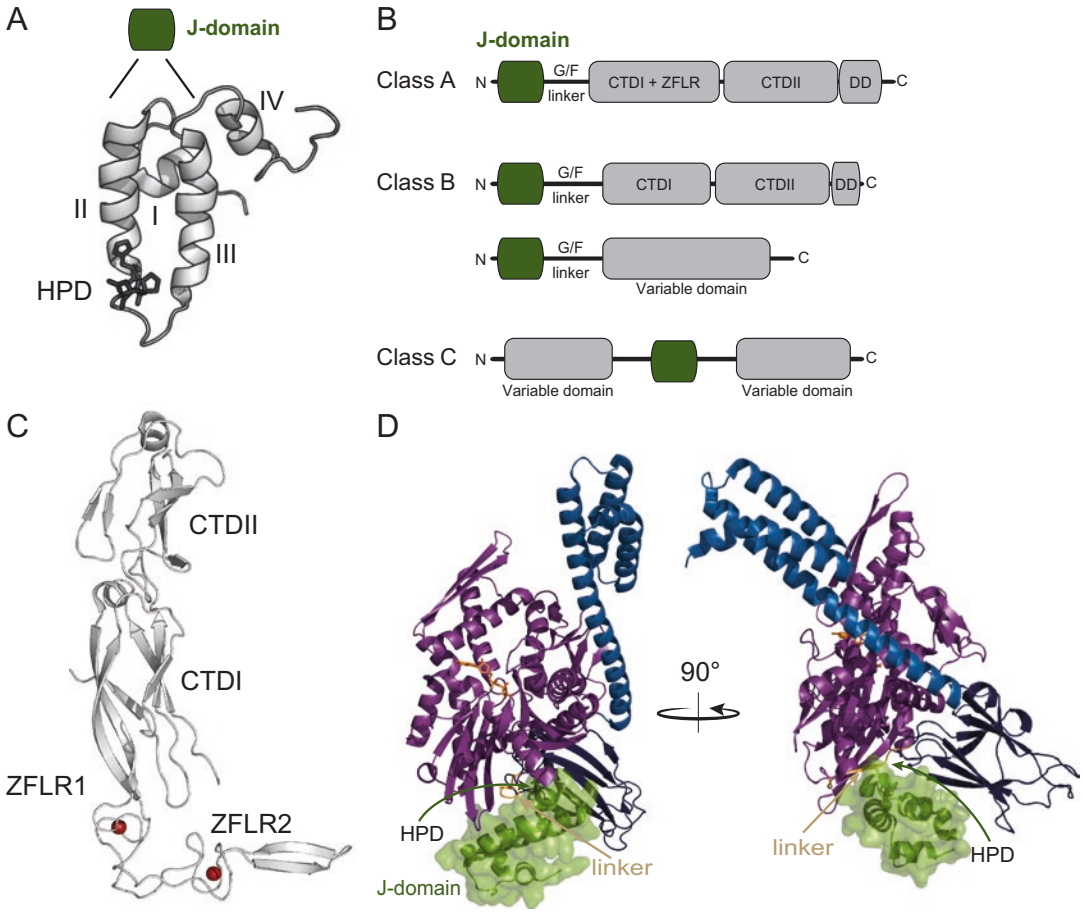

Fig. 1.2 Structure of J-domain proteins and their interaction with Hsp70s. (**a**) Cartoon representation of the J-domain structure of E. coli DnaJ (PDB ID 1XBL (Pellecchia et al. 1996)), highlighting the position of the conserved HPD motif. (**b**) General domain organization of class A (**top**), B (**middle**) and C (**bottom**) JDPs. The different domains are marked as follows: J, J-domain; G/F, Gly-Phe rich region; ZFLR, zinc-finger like region; CTD, C-terminal domain; DD, dimerization domain. (**c**) Cartoon representation of the Zn-binding and the C-terminal domains of S. cerevisiae Type I JDP, Ydj1 (PDB ID 1NLT (Li and Sha 2003)). (**d**) Structure of the J-domain of *E. coli* DnaJ in complex with *E. coli* DnaK, when the latter is in the ATP-bound open conformation (PDB ID 5NRO (Kityk et al. 2018)). The J-domain is shown in surface representation to highlight the contacts between residues of the J-domain and those of DnaK NBD (purple), SBDβ (dark blue), and the conserved linker (light orange)

of JDPs only with ATP-bound Hsp70s, as such an NBD-SBDβ interface is only formed in the ATP-bound conformation of Hsp70, and is broken upon ATP-hydrolysis. This structure also highlighted the fundamental importance of the conserved J-domain HPD motif in JDP activity, showing that the HPD interacts with key residues of the allosteric network in both the Hsp70 linker and NBD crevice, thereby arresting the NBD lobes and catalytic residues in a position optimal for ATP hydrolysis. The J-domain was further found to contact residues in the Hsp70 SBDβ that are connected to the signal pathway from the substrate to the NBD, making this transmission from the SBD to the NBD more efficient. In this manner, the J-domain couples two signals (Kityk et al. 2018) to trigger both ATP hydrolysis, and generate the ultrahigh affinity of the Hsp70 ADP-

bound equilibrium state (De Los Rios and Barducci 2014), leading to efficient trapping of substrates.

Moreover, residues in the J-domain that interact with DnaK, along with the corresponding residues in DnaK that interact with the J-domain, have been found to be highly conserved in evolution, suggesting that this mechanism is operational in all pairings of Hsp70s and J-domain proteins. Interestingly, the conserved J-domain sequences in different JDP paralogs which are thought to interact with the same Hsp70s, can deviate by several residues. This then raises the possibility that modulation within the J-domain sequences may establish a hierarchy of preferences between different JDPs and Hsp70s.

1.2.2 Nucleotide Exchange Factors

Although JDPs are considered the prime drivers of Hsp70 functional diversity, NEFs play an important role as well. The main function of NEFs is to help facilitate the exchange of ADP to ATP, which is done through a direct interaction with the Hsp70 NBD. By mediating the opening of the Hsp70 nucleotide binding cleft, NEFs facilitate the release of ADP, which in turn allows the rebinding of ATP and the subsequent release of substrates. Unlike the JDPs, which share a common J-domain, four evolutionarily unrelated families of NEFs have been identified with no sequence similarity among them. Although all NEFs interact with the Hsp70 NBD, each such family uses different mechanisms to open the Hsp70 nucleotide binding cleft for release of ADP.

In prokaryotes, mitochondria, and chloroplasts, nucleotide exchange in Hsp70s is regulated by GrpE, a homodimeric protein which consists of an N-terminal, unusually long α-helical dimerization domain, and a C-terminal β-sheet domain. The GrpE dimer interacts with a single Hsp70 molecule, inserting its β-sheet domain to literally drive a wedge into the nucleotide binding domain of Hsp70 (Harrison et al. 1997) (Fig. 1.3a, left). This complex formation induces a 14° rotation of subdomain IIB, resulting in an opening of the nucleotide binding cleft incompatible with nucleotide binding (Fig. 1.3a, right). As this interaction also induces asymmetry in the GrpE dimer, only one Hsp70 molecule can be bound at any given time.

The eukaryotic cytosol does not contain GrpE homologs, but rather nucleotide exchange is performed by three main classes of human NEFs: HspBP1, BAG proteins, and Hsp110s. While the classes are structurally distinct, with little or no homology between them, mechanistically, all seem to capture the open conformation of the Hsp70 NBD.

HspBP1/Sil1, which is found in the eukaryotic cytosol (HspBP1) and ER (Sil1), is composed entirely of four alpha-helical Armadillo repeats. The superhelical Sil1 protein wraps around subdomain IIB of the Hsp70 NBD (Fig. 1.3b, left) and rotates this subdomain around one of its helices (helix 7) (Yan et al. 2011) (Fig. 1.3b, right). Similarly to the role of GrpE in prokaryotes, the HspBP1-type NEFs seem to support the canonical chaperone actions of Hsp70 machines, from stress-related protein refolding to ER-associated degradation (ERAD) (Travers et al. 2000). HspBP1 also functionally resembles GrpE, in that both were shown to prevent unproductive rebinding of the released substrate (Gowda et al. 2018; Rosam et al. 2018), which occurs as dedicated structural elements within the NEFs that mimic motifs recognized by Hsp70s (e.g. RD of Fes1/HspBP1 (Gowda et al. 2018; Rosam et al. 2018) and possibly the N-terminal helical extension in GrpE (Harrison et al. 1997; Wu et al. 2012)) occupy the substrate binding sites on the chaperone (Fig. 1.3c).

BAG proteins form the second class of Hsp70 nucleotide exchange factors, and contain a conserved 110–124 residue long three-helix bundle BAG (Bcl2-associated athanogene) domain (Takayama et al. 1999) that binds to the subdomains IB and (mainly) IIB of Hsp70 NBD (Fig. 1.3d, left). This interaction of the BAG domain with Hsp70 locks the NBD in a conformation very similar to DnaK in complex with GrpE, with subdomain IIB tilted outward by 14°

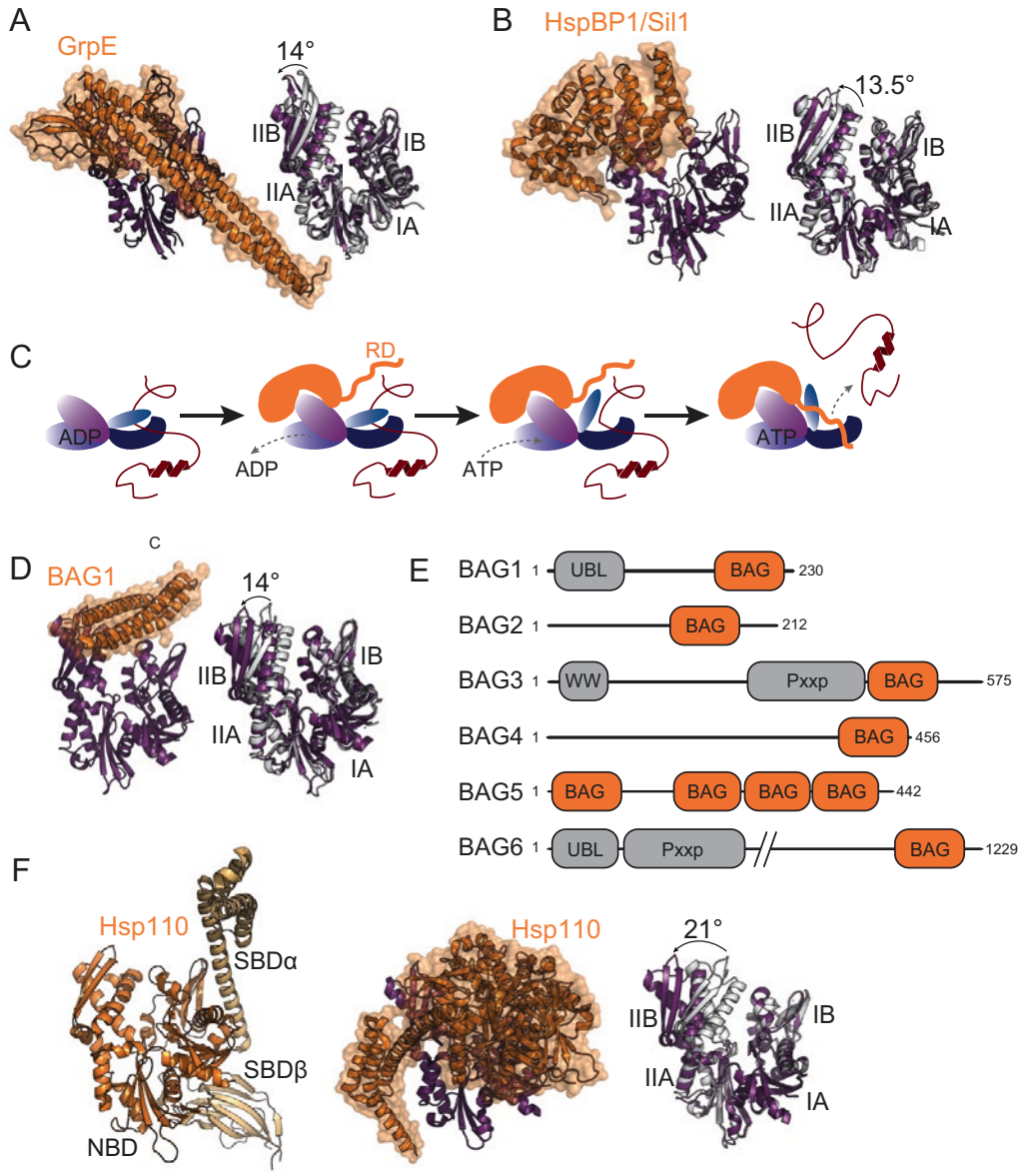

Fig. 1.3 Structures and mechanisms of nucleotide exchange factors. Exemplary structures of the four NEF families in complex with their respective Hsp70s, and the changes in the nucleotide binding domains of those Hsp70s following NEF binding. Crystal structures of (**a**) **Left:** GrpE in complex with DnaK-NBD (1DKG (Harrison et al. 1997)), (**a**) **Right:** Changes in DnaK NBD structure following GrpE binding (purple) overlaid on structure of unbound DnaK-NBD (white) to highlight the relative motion of the NBD I and II lobes upon NEF binding. (**b**) Same representations as in (A) for HspBP1 and Hsp70-NBD (3QML (Yan et al. 2011; Shomura et al. 2005)). (**c**) HspBP1 favors substrate release by preventing rebinding of the substrate after nucleotide exchange. From left to right: The Armadillo domain binds to subdomain IIB show to open the nucleotide binding cleft; after ATP binding, opening of the substrate binding cleft, and substrate dissociation; the N-terminal unstructured segment (RD, orange) of HspBP1 binds into the substrate binding pocket preventing rebinding of the substrate. (**d**) Structure of Bag1 in complex with Hcs70-NBD (1HX1 (Sondermann et al. 2001)), layout and colors are as in (A). (**e**) Domain organization of human BAG family proteins. All six reported BAG proteins contain a BAG domain at their C-terminus (orange). Some BAG proteins contain other domains, including the ubiquitin-like (UBL) domain, WW domain, and proline-rich regions (PXXP). Numbers to the right of the linear peptide sequence indicate lengths of the proteins. (*f*) Structural organization of Hsp110 NEF showing the homologies to Hsp70 NBD, SBD and Lid domains (left). Structure of Hsp110 (Sse1p; orange) in complex with Hsp70 (purple) (3D2F (Polier et al. 2008)) (middle) and an overlay of Hsp70 NBD structure with (purple) and without Hsp110 (white) to highlight the relative motion of the NBD I and II lobes upon Hsp110 binding (right)

(Sondermann et al. 2001) (Fig. 1.3d, right). In addition to this conserved domain, Bag domain proteins also contain a number of additional interaction domains, through which these NEFs can (1) be localized to specific subcellular structures, (2) ensure precise timing and targeting of nucleotide exchange, and (3) allow for timed transfer of Hsp70 substrates to other complexes (Fig. 1.3e). Such a mechanism, for example, can be found for BAG1, which contains an integral ubiquitin-like (UBL) domain that can serve as a proteasomal targeting signal, promoting the transfer of Hsp70-bound client proteins to the proteasome for degradation (Luders et al. 2000). Another NEF, BAG3, triggers the recruitment of the autophagic ubiquitin adaptor p62, and thus facilitates Hsp70-assisted substrate degradation through the autophagosome-lysosome pathway.

Hsp110 proteins, members of the third class of human NEFs, were initially grouped as Hsp70 family members because of similarities in sequence. Like Hsp70s, they consist of an N-terminal nucleotide binding domain (NBD) that is connected to a peptide-binding domain (in this case, a nine-stranded β-sandwich) by a flexible linker and an alpha-helical lid (SBDα) (Liu and Hendrickson 2007) (Fig. 1.3f, left). Hsp110s catalyze nucleotide exchange by a head-to-head interaction of their NBD with the NBD of Hsp70s, attaching to the side of subdomain IIB, while anchoring to the remainder of the NBD (Polier et al. 2008; Schuermann et al. 2008) (Fig. 1.3f, middle) and tilting subdomain IIB outward in a manner similar to GrpE (Fig. 1.3f, right). Intriguingly, some Hsp110s, similarly to Hsp70s, can directly bind unfolded proteins and prevent their aggregation. Moreover, Hsp110s are essential components in the human disaggregation machinery and cannot be replaced by any other class of NEF (Nillegoda et al. 2015). While Hsp110s display both structure and sequence similarity to canonical Hsp70s, and have even shown some level of ATPase activity, they cannot, however, employ a nucleotide-dependent, peptide-binding release cycle (Brodsky et al. 1999; Garcia et al. 2017).

1.2.2.1 Nucleotide Cycle Regulation Beyond JDPs and NEFs

Several factors have been identified, which, although not universal and thus not part of the core Hsp70 machinery, affect Hsp70 ATP hydrolysis and ADP release. The best studied factor, Hip (also known as p48), was identified over a decade ago as a protein that preferentially binds to and stabilizes the ADP-bound state of Hsp70 (Hohfeld et al. 1995). Hip competes with the BAG1 NEF for binding to Hsp70s NBD, thereby slowing down the nucleotide release and extending the time for which client proteins remain bound (Li et al. 2013). This prolonged substrate residence on Hsp70, mediated by Hip, may serve to prevent aggregation, as increased levels of Hip have been shown to reduce pathologic protein aggregation associated with Parkinson's disease (Roodveldt et al. 2009). Furthermore, through its tetratricopeptide repeat (TPR) domain, Hip can also coordinate between Hsp70s and additional cellular chaperones and factors. Thus, Hip protein may serve as an additional layer in the regulation of protein quality control, on top of the multiple isoforms of J proteins and different types of NEFs in the cell.

1.3 Hsp70s Interaction with Other Cellular Chaperone Systems

The Hsp70 chaperone machinery often doesn't act alone, but rather works in concert with other chaperone machines, such as the cytosolic AAA+ disaggregases, Hsp90 chaperones, and small heat shock proteins. This wide array of possible interactions generates a multitude of possible outcomes, where the mutually exclusive binding of Hsp70 to a specific co-chaperone essentially dictates the fate of its substrate.

One such example is the interaction of Hsp70, through its intrinsically disordered C-terminal EEVD amino acids, to TPR-domain co-chaperones, Hop and CHIP. Hop co-chaperone (Hsp70/Hsp90 organizing protein) can simulta-

neously bind Hsp70 (through its TRP1 domain) and Hsp90 (through the TPRA2 domain), thus serving as a bridge and facilitating the efficient transfer of substrates between these two chaperone systems (Scheufler et al. 2000). CHIP (carboxyl terminus of Hsc70 interacting protein) also interacts with the Hsp70 EEVD tail through its N-terminal TPR domain. However, unlike the folding function associated with Hop, the CHIP-Hsp70 interaction facilitates ubiquitination of Hsp70-bound substrates, thereby targeting these clients for proteasomal degradation (McDonough and Patterson 2003). Thus, when Hop and CHIP, through their TPR domains, compete for binding to the Hsp70 C-terminal tail, for the substrate it is truly a matter of life (folding) or death (degradation).

The conserved EEVD motif in the Hsp70 CTD has further been proposed to interact with additional proteins, including a receptor on the mitochondrial outer membrane (Li et al. 2009), and class B JDPs (Yu et al. 2015), hinting at further, yet to be discovered functions. Although the EEVD motif is present in all cytosolic eukaryotic Hsp70s, it is not found in the mitochondrial, or ER-resident Hsp70 isoforms, or in the prokaryotic DnaK. This raises many interesting questions about how and why the domain, and its associated functional versatility, evolved.

1.3.1 Hsp70 Chaperones in Protein Disaggregation

Another remarkable example of Hsp70 cooperating with, or rather being hijacked by, other cellular chaperones, is the protein disaggregation system. The seminal work on the yeast Hsp104 disaggregase from the Lindquist laboratory showed that Hsp104 chaperone, working in collaboration with the Hsp70 system, can disassemble aggregated proteins both *in vivo* (Parsell et al. 1994) and *in vitro* (Glover and Lindquist 1998). The Hsp104 hexamer is the main engine that couples ATP hydrolysis to generate the mechanical force needed for solubilizing protein aggregates, but it does not exhibit disaggregation activity on its own and strictly requires the cooperation of Hsp70 chaperone systems (Glover and Lindquist 1998; Mogk et al. 1999, 2015). The disaggregation reaction is, in fact, initiated by Hsp70 binding to the surface of protein aggregates, and only then followed by subsequent recruitment of the Hsp104 disaggregase (Winkler et al. 2012; Acebron et al. 2009). While Hsp70s can promiscuously bind to different kinds of protein aggregates, their disaggregation potential is very limited. In this sense, Hsp104s represent specialized Hsp70 partner chaperones that expand the capabilities of the Hsp70 machinery to include protein disaggregation. In addition to targeting Hsp104s to protein aggregates, Hsp70 activity is also required for activation of the substrate-unraveling function of the hexamer (Carroni et al. 2014; Rosenzweig et al. 2013). There, a direct interaction between Hsp70 NBD and the Hsp104 coiled-coil middle domains (CCDs) releases the repression of Hsp104 threading activity, which is caused by head-to-tail interactions of neighboring CCDs in the Hsp104 hexameric ring (Carroni et al. 2014; Oguchi et al. 2012; Heuck et al. 2016). Through this protective, Hsp70-regulated mechanism, nonspecific activation of Hsp104 is prevented, along with any deleterious and uncontrolled unfolding of cellular proteins (Oguchi et al. 2012; Lipinska et al. 2013). And while the Hsp70-Hsp104 interaction occurs at the same site in the Hsp70 NBD as NEF binding, the outcomes are entirely different – serving as a prime example of the versatility of the Hsp70 system, and how, through interaction with different cellular machineries, it can be tuned to very different cellular functions.

1.4 Conclusion and Perspectives

As illustrated in this text, the Hsp70 chaperone system plays a key part in a myriad of cellular functions, and considerable progress has been made in recent years in elucidating the structural and mechanistic basis for the chaperone functions and their interaction with co-chaperones. Despite this, though, many questions still remain. It is still unclear, for example, what is the role of the SBDα lid domain in the Hsp70-substrate

interaction. Further questions include how JDP co-chaperones target their Hsp70 partner proteins to substrates, as well as the extent of substrate remodeling performed by the JDPs themselves. Moreover, with such a wide array of possible Hsp70 functions, perhaps the most important unresolved issues regarding this mechanism are what factors govern the pathway decisions which direct Hsp70 substrates to sequestration, refolding, or degradation by the ubiquitin proteasome system and autophagy; and critically, how various stress and disease states impact these vital decisions.

Acknowledgments R.R. is supported by the European Research Council starting grant (ERC-2018-STG 802001), the Azrieli Foundation, and a research grant from the Blythe Brenden-Mann New Scientist Fund.

References

Abrams JL, Verghese J, Gibney PA, Morano KA (2014) Hierarchical functional specificity of cytosolic heat shock protein 70 (Hsp70) nucleotide exchange factors in yeast. J Biol Chem 289:13155–13167

Acebron SP, Martin I, del Castillo U, Moro F, Muga A (2009) DnaK-mediated association of ClpB to protein aggregates. A bichaperone network at the aggregate surface. FEBS Lett 583:2991–2996

Alderson TR, Kim JH, Cai K, Frederick RO, Tonelli M, Markley JL (2014) The specialized Hsp70 (HscA) interdomain linker binds to its nucleotide-binding domain and stimulates ATP hydrolysis in both cis and trans configurations. Biochemistry 53:7148–7159

Balchin D, Hayer-Hartl M, Hartl FU (2016) In vivo aspects of protein folding and quality control. Science 353:aac4354

Banerjee R, Jayaraj GG, Peter JJ, Kumar V, Mapa K (2016) Monitoring conformational heterogeneity of the lid of DnaK substrate-binding domain during its chaperone cycle. FEBS J 283:2853–2868

Bertelsen EB, Chang L, Gestwicki JE, Zuiderweg ER (2009) Solution conformation of wild-type E. coli Hsp70 (DnaK) chaperone complexed with ADP and substrate. Proc Natl Acad Sci U S A 106:8471–8476

Bocking T, Aguet F, Harrison SC, Kirchhausen T (2011) Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. Nat Struct Mol Biol 18:295–301

Bracher A, Verghese J (2015) The nucleotide exchange factors of Hsp70 molecular chaperones. Front Mol Biosci 2:10

Brodsky JL, Werner ED, Dubas ME, Goeckeler JL, Kruse KB, McCracken AA (1999) The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J Biol Chem 274:3453–3460

Carroni M, Kummer E, Oguchi Y, Wendler P, Clare DK, Sinning I, Kopp J, Mogk A, Bukau B, Saibil HR (2014) Head-to-tail interactions of the coiled-coil domains regulate ClpB activity and cooperation with Hsp70 in protein disaggregation. Elife (Cambridge) 3:e02481

Chakraborty A, Mukherjee S, Chattopadhyay R, Roy S, Chakrabarti S (2014) Conformational adaptation in the E. coli sigma 32 protein in response to heat shock. J Phys Chem B 118:4793–4802

Chang YW, Sun YJ, Wang C, Hsiao CD (2008) Crystal structures of the 70-kDa heat shock proteins in domain disjoining conformation. J Biol Chem 283:15502–15511

Cheetham ME, Caplan AJ (1998) Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. Cell Stress Chaperones 3:28–36

Clerico EM, Tilitsky JM, Meng W, Gierasch LM (2015) How Hsp70 molecular machines interact with their substrates to mediate diverse physiological functions. J Mol Biol 427:1575–1588

Clerico EM, Meng W, Pozhidaeva A, Bhasne K, Petridis C, Gierasch LM (2019) Hsp70 molecular chaperones: multifunctional allosteric holding and unfolding machines. Biochem J 476:1653–1677

Craig EA (2018) Hsp70 at the membrane: driving protein translocation. BMC Biol 16:11

Cupp-Vickery JR, Peterson JC, Ta DT, Vickery LE (2004) Crystal structure of the molecular chaperone HscA substrate binding domain complexed with the IscU recognition peptide ELPPVKIHC. J Mol Biol 342:1265–1278

De Los Rios P, Barducci A (2014) Hsp70 chaperones are non-equilibrium machines that achieve ultra-affinity by energy consumption. Elife 3:e02218

Doring K, Ahmed N, Riemer T, Suresh HG, Vainshtein Y, Habich M, Riemer J, Mayer MP, O'Brien EP, Kramer G, Bukau B (2017) Profiling Ssb-nascent chain interactions reveals principles of Hsp70-assisted folding. *Cell* 170:298–311 e220

Dutkiewicz R, Schilke B, Cheng S, Knieszner H, Craig EA, Marszalek J (2004) Sequence-specific interaction between mitochondrial Fe-S scaffold protein Isu and Hsp70 Ssq1 is essential for their in vivo function. J Biol Chem 279:29167–29174

Fernandez-Fernandez MR, Gragera M, Ochoa-Ibarrola L, Quintana-Gallardo L, Valpuesta JM (2017) Hsp70 – a master regulator in protein degradation. FEBS Lett 591:2648–2660

Finka A, Sood V, Quadroni M, Rios Pde L, Goloubinoff P (2015) Quantitative proteomics of heat-treated human cells show an across-the-board mild depletion

of housekeeping proteins to massively accumulate few HSPs. Cell Stress Chaperones 20:605–620

Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three-dimensional structure of the ATPase fragment of a 70K heat-shock cognate protein. Nature 346:623–628

Fotin A, Cheng Y, Sliz P, Grigorieff N, Harrison SC, Kirchhausen T, Walz T (2004) Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature 432:573–579

Fourie AM, Sambrook JF, Gething MJ (1994) Common and divergent peptide binding specificities of hsp70 molecular chaperones. J Biol Chem 269:30470–30478

Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu Rev Biochem 70:603–647

Garcia VM, Nillegoda NB, Bukau B, Morano KA (2017) Substrate binding by the yeast Hsp110 nucleotide exchange factor and molecular chaperone Sse1 is not obligate for its biological activities. Mol Biol Cell 28:2066–2075

Glover JR, Lindquist S (1998) Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell 94:73–82

Goloubinoff P, De Los Rios P (2007) The mechanism of Hsp70 chaperones: (entropic) pulling the models together. Trends Biochem Sci 32:372–380

Gowda NKC, Kaimal JM, Kityk R, Daniel C, Liebau J, Ohman M, Mayer MP, Andreasson C (2018) Nucleotide exchange factors Fes1 and HspBP1 mimic substrate to release misfolded proteins from Hsp70. Nat Struct Mol Biol 25:83–89

Gragerov A, Gottesman ME (1994) Different peptide binding specificities of hsp70 family members. J Mol Biol 241:133–135

Hageman J, Rujano MA, van Waarde MA, Kakkar V, Dirks RP, Govorukhina N, Oosterveld-Hut HM, Lubsen NH, Kampinga HH (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. Mol Cell 37:355–369

Harrison CJ, Hayer-Hartl M, Di Liberto M, Hartl F, Kuriyan J (1997) Crystal structure of the nucleotide exchange factor GrpE bound to the ATPase domain of the molecular chaperone DnaK. Science 276:431–435

Heuck A, Schitter-Sollner S, Suskiewicz MJ, Kurzbauer R, Kley J, Schleiffer A, Rombaut P, Herzog F, Clausen T (2016) Structural basis for the disaggregase activity and regulation of Hsp104. Elife 5:pii: e21516

Hoff KG, Ta DT, Tapley TL, Silberg JJ, Vickery LE (2002) Hsc66 substrate specificity is directed toward a discrete region of the iron-sulfur cluster template protein IscU. J Biol Chem 277:27353–27359

Hohfeld J, Minami Y, Hartl FU (1995) Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. Cell 83:589–598

Jiang J, Prasad K, Lafer EM, Sousa R (2005) Structural basis of interdomain communication in the Hsc70 chaperone. Mol Cell 20:513–524

Kakkar V, Mansson C, de Mattos EP, Bergink S, van der Zwaag M, van Waarde M, Kloosterhuis NJ, Melki R, van Cruchten RTP, Al-Karadaghi S, Arosio P, Dobson CM, Knowles TPJ, Bates GP, van Deursen JM, Linse S, van de Sluis B, Emanuelsson C, Kampinga HH (2016) The S/T-rich motif in the DNAJB6 chaperone delays polyglutamine aggregation and the onset of disease in a mouse model. Mol Cell 62:272–283

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592

Kellner R, Hofmann H, Barducci A, Wunderlich B, Nettels D, Schuler B (2014) Single-molecule spectroscopy reveals chaperone-mediated expansion of substrate protein. Proc Natl Acad Sci U S A 111:13355–13360

Kityk R, Kopp J, Sinning I, Mayer MP (2012) Structure and dynamics of the ATP-bound open conformation of Hsp70 chaperones. Mol Cell 48:863–874

Kityk R, Vogel M, Schlecht R, Bukau B, Mayer MP (2015) Pathways of allosteric regulation in Hsp70 chaperones. Nat Commun 6:8308

Kityk R, Kopp J, Mayer MP (2018) Molecular mechanism of J-domain-triggered ATP hydrolysis by Hsp70 chaperones. Mol Cell 69:227–237 e224

Kluck CJ, Patzelt H, Genevaux P, Brehmer D, Rist W, Schneider-Mergener J, Bukau B, Mayer MP (2002) Structure-function analysis of HscC, the Escherichia coli member of a novel subfamily of specialized Hsp70 chaperones. J Biol Chem 277:41060–41069

Kramer G, Boehringer D, Ban N, Bukau B (2009) The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins. Nat Struct Mol Biol 16:589–597

Lee JH, Zhang D, Hughes C, Okuno Y, Sekhar A, Cavagnero S (2015) Heterogeneous binding of the SH3 client protein to the DnaK molecular chaperone. Proc Natl Acad Sci U S A 112:E4206–E4215

Lee K, Sharma R, Shrestha OK, Bingman CA, Craig EA (2016) Dual interaction of the Hsp70 J-protein cochaperone Zuotin with the 40S and 60S ribosomal subunits. Nat Struct Mol Biol 23:1003–1010

Li J, Sha B (2003) Preliminary X-ray crystallographic studies of yeast Hsp40 Ydj1 complexed with its peptide substrate. Acta Crystallogr D Biol Crystallogr 59:1317–1319

Li J, Qian X, Hu J, Sha B (2009) Molecular chaperone Hsp70/Hsp90 prepares the mitochondrial outer membrane translocon receptor Tom71 for preprotein loading. J Biol Chem 284:23852–23859

Li Z, Hartl FU, Bracher A (2013) Structure and function of Hip, an attenuator of the Hsp70 chaperone cycle. Nat Struct Mol Biol 20:929–935

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191

Linke K, Wolfram T, Bussemer J, Jakob U (2003) The roles of the two zinc binding sites in DnaJ. J Biol Chem 278:44457–44466

Lipinska N, Zietkiewicz S, Sobczak A, Jurczyk A, Potocki W, Morawiec E, Wawrzycka A, Gumowski K, Slusarz M, Rodziewicz-Motowidlo S, Chrusciel E, Liberek K (2013) Disruption of ionic interactions between the nucleotide binding domain 1 (NBD1) and middle (M) domain in Hsp100 disaggregase unleashes toxic hyperactivity and partial independence from Hsp70. J Biol Chem 288:2857–2869

Liu Q, Hendrickson WA (2007) Insights into Hsp70 chaperone activity from a crystal structure of the yeast Hsp110 Sse1. Cell 131:106–120

Luders J, Demand J, Papp O, Hohfeld J (2000) Distinct isoforms of the cofactor BAG-1 differentially affect Hsc70 chaperone function. J Biol Chem 275:14817–14823

Marcinowski M, Holler M, Feige MJ, Baerend D, Lamb DC, Buchner J (2011) Substrate discrimination of the chaperone BiP by autonomous and cochaperone-regulated conformational transitions. Nat Struct Mol Biol 18:150–158

Marcinowski M, Rosam M, Seitz C, Elferich J, Behnke J, Bello C, Feige MJ, Becker CF, Antes I, Buchner J (2013) Conformational selection in substrate recognition by Hsp70 chaperones. J Mol Biol 425:466–474

Mashaghi A, Bezrukavnikov S, Minde DP, Wentink AS, Kityk R, Zachmann-Brand B, Mayer MP, Kramer G, Bukau B, Tans SJ (2016) Alternative modes of client binding enable functional plasticity of Hsp70. Nature 539:448–451

Mayer MP (2013) Hsp70 chaperone dynamics and molecular mechanism. Trends Biochem Sci 38:507–514

Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684

Mayer MP, Kityk R (2015) Insights into the molecular mechanism of allostery in Hsp70s. Front Mol Biosci 2:58

McDonough H, Patterson C (2003) CHIP: a link between the chaperone and proteasome systems. Cell Stress Chaperones 8:303–308

Meyer AE, Hung NJ, Yang P, Johnson AW, Craig EA (2007) The specialized cytosolic J-protein, Jjj1, functions in 60S ribosomal subunit biogenesis. Proc Natl Acad Sci U S A 104:1558–1563

Mogk A, Tomoyasu T, Goloubinoff P, Rudiger S, Roder D, Langen H, Bukau B (1999) Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB. EMBO J 18:6934–6949

Mogk A, Kummer E, Bukau B (2015) Cooperation of Hsp70 and Hsp100 chaperone machines in protein disaggregation. Front Mol Biosci 2:22

Mogk A, Bukau B, Kampinga HH (2018) Cellular handling of protein aggregates by disaggregation machines. Mol Cell 69:214–226

Mok SA, Condello C, Freilich R, Gillies A, Arhar T, Oroz J, Kadavath H, Julien O, Assimon VA, Rauch JN, Dunyak BM, Lee J, Tsai FTF, Wilson MR, Zweckstetter M, Dickey CA, Gestwicki JE (2018) Mapping interactions with the chaperone network reveals factors that protect against tau aggregation. Nat Struct Mol Biol 25:384–393

Morshauser RC, Wang H, Flynn GC, Zuiderweg ER (1995) The peptide-binding domain of the chaperone protein Hsc70 has an unusual secondary structure topology. Biochemistry 34:6261–6266

Morshauser RC, Hu W, Wang H, Pang Y, Flynn GC, Zuiderweg ER (1999) High-resolution solution structure of the 18 kDa substrate-binding domain of the mammalian chaperone protein Hsc70. J Mol Biol 289:1387–1403

Nillegoda NB, Kirstein J, Szlachcic A, Berynskyy M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R, Guilbride DL, Wade RC, Morimoto RI, Mayer MP, Bukau B (2015) Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature 524:247–251

Nillegoda NB, Stank A, Malinverni D, Alberts N, Szlachcic A, Barducci A, De Los Rios P, Wade RC, Bukau B (2017) Evolution of an intricate J-protein network driving protein disaggregation in eukaryotes. Elife 6:pii: e24560

Nillegoda NB, Wentink AS, Bukau B (2018) Protein disaggregation in multicellular organisms. Trends Biochem Sci 43:285–300

Oguchi Y, Kummer E, Seyffer F, Berynskyy M, Anstett B, Zahn R, Wade RC, Mogk A, Bukau B (2012) A tightly regulated molecular toggle controls AAA+ disaggregase. Nat Struct Mol Biol 19:1338–1346

Palleros DR, Shi L, Reid KL, Fink AL (1994) hsp70-protein complexes. Complex stability and conformation of bound substrate protein. J Biol Chem 269:13107–13114

Parsell DA, Kowal AS, Singer MA, Lindquist S (1994) Protein disaggregation mediated by heat-shock protein Hsp104. Nature 372:475–478

Pellecchia M, Szyperski T, Wall D, Georgopoulos C, Wuthrich K (1996) NMR structure of the J-domain and the Gly/Phe-rich region of the Escherichia coli DnaJ chaperone. J Mol Biol 260:236–250

Pellecchia M, Montgomery DL, Stevens SY, Vander Kooi CW, Feng HP, Gierasch LM, Zuiderweg ER (2000) Structural insights into substrate binding by the molecular chaperone DnaK. Nat Struct Biol 7:298–303

Polier S, Dragovic Z, Hartl FU, Bracher A (2008) Structural basis for the cooperation of Hsp70 and Hsp110 chaperones in protein folding. Cell 133:1068–1079

Preissler S, Deuerling E (2012) Ribosome-associated chaperones as key players in proteostasis. Trends Biochem Sci 37:274–283

Qu B, Jia Y, Liu Y, Wang H, Ren G (2015) The detection and role of heat shock protein 70 in various nondisease conditions and disease conditions: a literature review. Cell Stress Chaperones 20:885–892

Rapoport I, Boll W, Yu A, Bocking T, Kirchhausen T (2008) A motif in the clathrin heavy chain required for the Hsc70/auxilin uncoating reaction. Mol Biol Cell 19:405–413

Rodriguez F, Arsene-Ploetze F, Rist W, Rudiger S, Schneider-Mergener J, Mayer MP, Bukau B (2008) Molecular basis for regulation of the heat shock transcription factor sigma32 by the DnaK and DnaJ chaperones. Mol Cell 32:347–358

Roodveldt C, Bertoncini CW, Andersson A, van der Goot AT, Hsu ST, Fernandez-Montesinos R, de Jong J, van Ham TJ, Nollen EA, Pozo D, Christodoulou J, Dobson CM (2009) Chaperone proteostasis in Parkinson's disease: stabilization of the Hsp70/alpha-synuclein complex by Hip. EMBO J 28:3758–3770

Rosam M, Krader D, Nickels C, Hochmair J, Back KC, Agam G, Barth A, Zeymer C, Hendrix J, Schneider M, Antes I, Reinstein J, Lamb DC, Buchner J (2018) Bap (Sil1) regulates the molecular chaperone BiP by coupling release of nucleotide and substrate. Nat Struct Mol Biol 25:90–100

Rosenzweig R, Moradi S, Zarrine-Afsar A, Glover JR, Kay LE (2013) Unraveling the mechanism of protein disaggregation through a ClpB-DnaK interaction. Science 339:1080–1083

Rosenzweig R, Sekhar A, Nagesh J, Kay LE (2017) Promiscuous binding by Hsp70 results in conformational heterogeneity and fuzzy chaperone-substrate ensembles. Elife 6:pii: e28030

Rosenzweig R, Nillegoda NB, Mayer MP, Bukau B (2019) The Hsp70 chaperone network. Nat Rev Mol Cell Biol 20:665–680

Rudiger S, Germeroth L, Schneider-Mergener J, Bukau B (1997) Substrate specificity of the DnaK chaperone determined by screening cellulose-bound peptide libraries. EMBO J 16:1501–1507

Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 101:199–210

Schlecht R, Erbse AH, Bukau B, Mayer MP (2011) Mechanics of Hsp70 chaperones enables differential interaction with client proteins. Nat Struct Mol Biol 18:345–351

Schuermann JP, Jiang J, Cuellar J, Llorca O, Wang L, Gimenez LE, Jin S, Taylor AB, Demeler B, Morano KA, Hart PJ, Valpuesta JM, Lafer EM, Sousa R (2008) Structure of the Hsp110:Hsc70 nucleotide exchange machine. Mol Cell 31:232–243

Sekhar A, Rosenzweig R, Bouvignies G, Kay LE (2015) Mapping the conformation of a client protein through the Hsp70 functional cycle. Proc Natl Acad Sci U S A 112:10395–10400

Sekhar A, Rosenzweig R, Bouvignies G, Kay LE (2016) Hsp70 biases the folding pathways of client proteins. Proc Natl Acad Sci U S A 113:E2794–E2801

Sekhar A, Velyvis A, Zoltsman G, Rosenzweig R, Bouvignies G, Kay LE (2018) Conserved conformational selection mechanism of Hsp70 chaperone-substrate interactions. Elife 7:pii: e32764

Shomura Y, Dragovic Z, Chang HC, Tzvetkov N, Young JC, Brodsky JL, Guerriero V, Hartl FU, Bracher A (2005) Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. Mol Cell 17:367–379

Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, Moarefi I (2001) Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science 291:1553–1557

Stevens SY, Cai S, Pellecchia M, Zuiderweg ER (2003) The solution structure of the bacterial HSP70 chaperone protein domain DnaK(393–507) in complex with the peptide NRLLLTG. Protein Sci 12:2588–2596

Szabo A, Korszun R, Hartl FU, Flanagan J (1996) A zinc finger-like domain of the molecular chaperone DnaJ is involved in binding to denatured protein substrates. EMBO J 15:408–417

Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC, Lindquist S (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. Cell 158:434–448

Takayama S, Xie Z, Reed JC (1999) An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J Biol Chem 274:781–786

Tapley TL, Cupp-Vickery JR, Vickery LE (2005) Sequence-dependent peptide binding orientation by the molecular chaperone DnaK. Biochemistry 44:12307–12315

Tiwari S, Kumar V, Jayaraj GG, Maiti S, Mapa K (2013) Unique structural modulation of a non-native substrate by cochaperone DnaJ. Biochemistry 52:1011–1018

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101:249–258

Tsai J, Douglas MG (1996) A conserved HPD sequence of the J-domain is necessary for YDJ1 stimulation of Hsp70 ATPase activity at a site distinct from substrate binding. J Biol Chem 271:9347–9354

Vogel M, Bukau B, Mayer MP (2006) Allosteric regulation of Hsp70 chaperones by a proline switch. Mol Cell 21:359–367

Walther DM, Kasturi P, Zheng M, Pinkert S, Vecchi G, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M, Mann M, Hartl FU (2015) Widespread proteome remodeling and aggregation in aging C. elegans. Cell 161:919–932

Willmund F, del Alamo M, Pechmann S, Chen T, Albanese V, Dammer EB, Peng J, Frydman J (2013) The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. Cell 152:196–209

Winkler J, Tyedmers J, Bukau B, Mogk A (2012) Hsp70 targets Hsp100 chaperones to substrates for protein disaggregation and prion fragmentation. J Cell Biol 198:387–404

Wu CC, Naveen V, Chien CH, Chang YW, Hsiao CD (2012) Crystal structure of DnaK protein complexed with nucleotide exchange factor GrpE in DnaK chaperone system: insight into intermolecular communication. J Biol Chem 287:21461–21470

Yan M, Li J, Sha B (2011) Structural analysis of the Sil1-Bip complex reveals the mechanism for Sil1 to function as a nucleotide-exchange factor. Biochem J 438:447–455

Yu HY, Ziegelhoffer T, Osipiuk J, Ciesielski SJ, Baranowski M, Zhou M, Joachimiak A, Craig EA (2015) Roles of intramolecular and intermolecular interactions in functional regulation of the Hsp70 J-protein co-chaperone Sis1. J Mol Biol 427:1632–1643

Zahn M, Berthold N, Kieslich B, Knappe D, Hoffmann R, Strater N (2013) Structural studies on the forward and reverse binding modes of peptides to the chaperone DnaK. J Mol Biol 425:2463–2479

Zhang Y, Sinning I, Rospert S (2017) Two chaperones locked in an embrace: structure and function of the ribosome-associated complex RAC. Nat Struct Mol Biol 24:611–619

Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrickson WA (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. Science 272:1606–1614

Zhuravleva A, Gierasch LM (2011) Allosteric signal transmission in the nucleotide-binding domain of 70-kDa heat shock protein (Hsp70) molecular chaperones. Proc Natl Acad Sci U S A 108:6987–6992

The TRiC/CCT Chaperonin and Its Role in Uncontrolled Proliferation

2

Dan Yang Wang, Kamila Kamuda, Guillermo Montoya, and Pablo Mesa

Abstract

The cell cycle is a sophisticated space-time regulated mechanism where a wide variety of protein modules and complexes associate functioning in a concerted manner to regulate and transfer the genetic material to daughter cells. CCT (chaperonin containing TCP-1, also known as TRiC) is a molecular machine that forms a high molecular weight complex (1000 KDa). CCT is emerging as a key molecule during mitosis due to its essential role in the folding of many important proteins involved in cell division (Cdh1, Plk1, p27, Cdc20, PP2a regulatory subunits, tubulin or actin) suggesting its involvement in uncontrolled proliferation. The assembly is formed by eight different subunits called CCTα, β, γ, δ, ε, ζ, η and θ in mammals corresponding to CCT1–8 in yeast. CCT/TRiC is organized in a unique intra- and inter-ring arrangement. The chaperonin monomers share a common domain structure including an equatorial domain, which contains all the inter-ring contacts, most of the intra-ring contacts and the ATP binding site, whose binding and hydrolysis triggers the conformational changes that take place during the functional cycle. All chaperonins display an open substrate-receptive conformation, where the unfolded protein is recognized and trapped, and a closed conformation where the substrate is isolated from the bulk of the intracellular environment. In this chapter we discuss the complex set of intra- and inter-ring allosteric signals during chaperonin function.

Keywords

Chaperonines · Molecular machines · Cell cycle · Protein folding · Allosterism · ATP hydrolysis · Protein-protein interaction · X-ray · cryoEM

2.1 Introduction

The synthesis of a protein begins with the transcription of the gene coding for the polypeptide to produce an mRNA, which can undergo splicing in eukaryotes to code for different protein isoforms. The mRNA is then translated into a polypeptide in the ribosome. These processes constitute the central dogma of molecular biology, and together with the replication of the genome, make the foundation of life. To perform their specific functions, synthesized proteins need to adopt their three-dimensional folded

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_2

structure. As the polypeptide chain is synthesized by the ribosome, it will start to fold into its native structure spontaneously in a process called co-translational folding, which is essential to avoid premature interactions between initially and temporally exposed regions of the protein and the cytosolic environment (Netzer and Hartl 1997; Nicola et al. 1999). A given protein may fold semi-autonomously, guided by a plethora of physicochemical forces including hydrophobic interactions, intramolecular hydrogen bonds, van der Waals forces, etc. (Dobson et al. 1998). However, certain nascent polypeptides are not able to fold into their native state, especially large proteins that contain multidomain structures. The exposed hydrophobic regions tend to aggregate in the crowded cellular environment, and the failure of achieving a native structure will produce an inactive protein or even a misfolded one with deleterious effects for the cell that can potentially cause severe diseases (Dobson et al. 1998; Kim et al. 2013). Molecular chaperones are a family of proteins that assist other proteins to fold into their native three-dimensional states – without being part of their final structures – and they are involved in many aspects of protein homeostasis including translocation, degradation and disaggregation (Hartl 1996; Kim et al. 2013).

Chaperonins are a class of cylindrical-shaped molecular chaperones with highly related sequences (Hemmingsen et al. 1988). In contrast to the majority of molecular chaperones, which perform many functions from folding, assembly and degradation, chaperonins have a more specific function, assisting the folding of newly-synthesized peptides. They are usually cylindrical barrels with hydrophobic inner chambers, designed to facilitate the folding process of protein substrates, powered by ATP hydrolysis, apart from the crowded cellular surroundings. These large protein complexes (840–950 kDa) have two rings stacked opposite to each other forming two substrate binding cavities. Each ring is composed of 7–9 subunits and its composition varies from homo-oligomers to hetero-oligomers (Gutsche et al. 1999; Horwich et al. 2007). Chaperonins are universal in eubacteria, archaea and eukaryotes, illustrating the importance of these large complexes (Yébenes et al. 2011). On the basis of their amino acid sequence homologies, chaperonins have been categorized into two groups, group I and group II (Gupta 1990). Group I chaperonins (*e.g.* GroEL) rely on a separate lid (*e.g.* GroES) to confine the substrates in their inner binding chambers, and they are found in eubacteria, mitochondria and chloroplasts. Group II chaperonins, ubiquitous in archaea (Thermosomes) and eukarya, feature a distinctive built-in protrusion with the same structural encapsulating function (Kubota et al. 1994; Trent et al. 1991; Valpuesta et al. 2005). In this chapter, we will mainly focus on the most complex group II chaperonin, the eukaryotic cytosolic chaperonin TRiC (T-complex protein-1 ring complex), also known as CCT (chaperonin containing TCP-1).

2.2 The Structure of CCT

The CCT complex is composed of two sets of 8 different subunits (Lewis et al. 1992). These two sets form identical heterologous octameric rings stacked back to back to form two central binding cavities where the folding process takes place (Fig. 2.1a, b). The 8 different subunits of CCT contain dissimilar homologous sequences but share similar structures. Each subunit contains apical, intermediate and equatorial domains (Fig. 2.1c) (Ditzel et al. 1998). The most conserved region is the equatorial domain, which contains the ATP binding site and most of the intra- and inter-ring contacts. The binding and hydrolysis of ATP and the release of its products (ADP and Pi) induce the conformational changes that power the folding process, with the whole complex alternating between an open and a closed conformation (Douglas et al. 2011; Llorca et al. 1998; Meyer et al. 2003). Apical domains are the least homologous regions and have been proposed to recognize, interact with and capture the substrates, thus initiating the folding process. The helical protrusions, located at the tip of apical domains, function as a built-in lid involved in the closure of the inner chambers to confine the

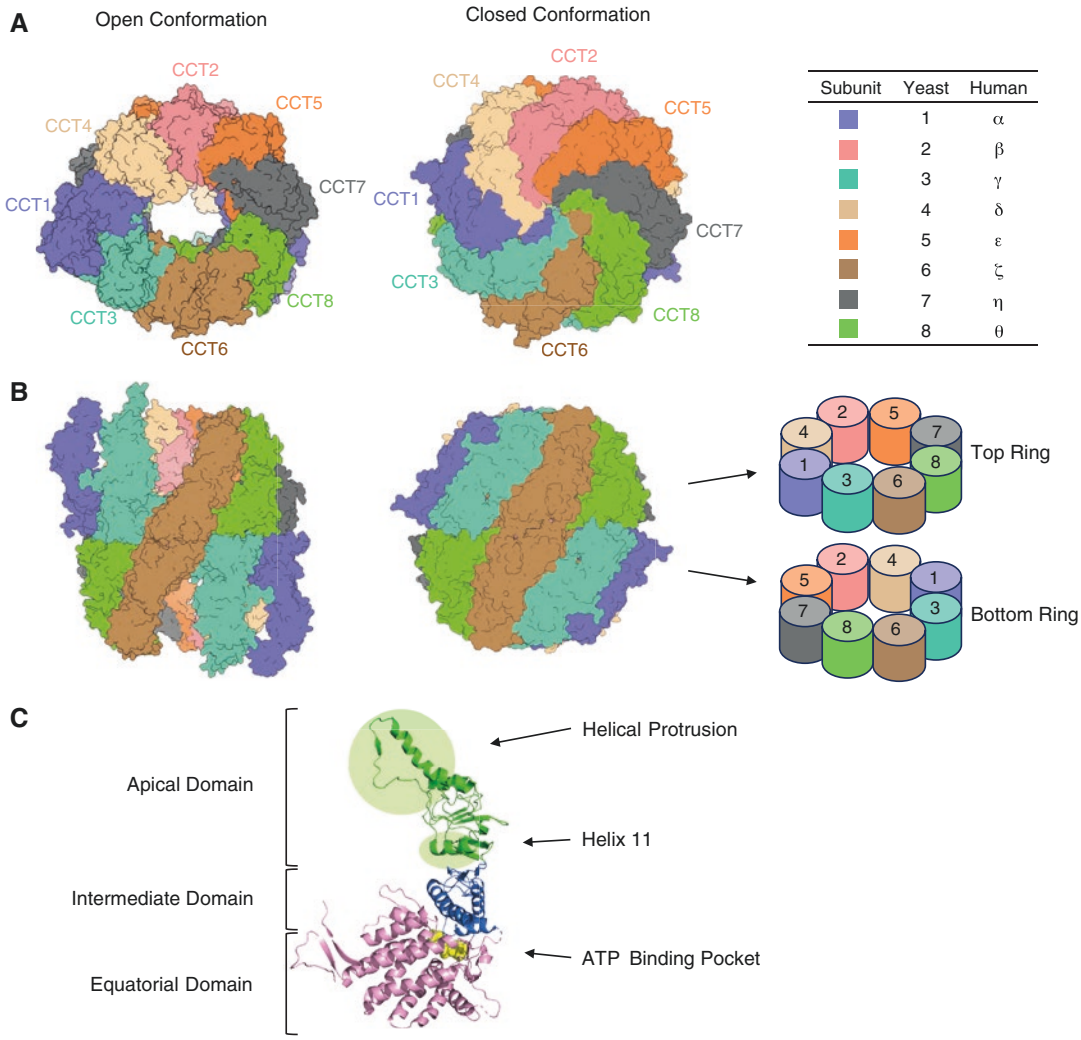

Fig. 2.1 Molecular architecture of CCT. (**a**) Top views, using a surface representation, of the open and closed conformations of yeast CCT (left, PDB ID 5GW5, and right, PDB ID 4AOL, respectively). The color code of the different subunits and its nomenclature for yeast and human is indicated on the right panel. The arrangement of subunits is CCT1, CCT3, CCT6, CCT8, CCT7, CCT5, CCT2, CCT4 (using yeast nomenclature; the human counterpart is CCTα, CCTγ, CCTζ, CCTθ, CCTη, CCTε, CCTβ, CCTδ). (**b**) Side views of the same conformations. The eight different CCT subunits assemble in a common and specific order into two rings stacked back to back, where subunits CCT2 and CCT6 from one ring contact equivalent subunits on the other ring. An sketch representation of both rings is shown, at the right panel, to highlight the inter-ring contacts of the monomers. (**c**) The structure of CCT1 is shown as a representative subunit, containing the apical (green), intermediate (blue) and equatorial domains (pink). The helical protrusion, Helix 11 and the ATP binding pocket (with ADP depicted in yellow) are pointed by arrows

substrate (Booth et al. 2008; Klumpp et al. 1997). NMR studies indicated that the substrate binding site includes alfa-helix 11 of the apical domain, which corresponds to helix I in the substrate binding site of GroEL (Ditzel et al. 1998; Joachimiak et al. 2014). The intermediate domain would work as a molecular hinge that bidirectionally transmits and integrates the different conformational changes at the apical and the

equatorial domains (Muñoz et al. 2011; Yébenes et al. 2011).

Although CCT has been studied over decades, the exact arrangement of its constituent 8 subunits has been a matter of debate for a long time. Recently, Kalisman et al. and Leitner et al. employed cross-linking, mass spectrometry and combinatorial homology modelling to propose a specific subunit order for both CCT rings, showed in Fig. 2.1c (Kalisman et al. 2012; Leitner et al. 2012). This organizational pattern has been confirmed by cryo-EM studies in which an eGFP label was inserted into specific subunits (Zang et al. 2016, 2018). The first structure of the CCT open conformation, solved by X-ray crystallography (Muñoz et al. 2011), showed that the different apical domains presented diverse positionings, with the apical face of subunit α on the most extreme and external orientation (Fig. 2.1a, b). This structural characteristic, which confers an asymmetrical shape to the complex, was confirmed by cryoEM studies (Zhang et al. 2016), and it could represent a receptive state able to interact with the substrate proteins and where the apical domains have their movement restrained.

2.3 CCT Substrates and Specificity

The first cellular function of the eukaryotic CCT chaperonin was linked exclusively with the folding of cytoskeletal proteins tubulin and actin (Vinh and Drubin 1994; Yaffe et al. 1992) during their biogenesis. More candidates for chaperonin substrates were then identified by the systematic analysis of yeast proteome by high-throughput mass spectrometry (Gavin et al. 2002; Ho et al. 2002). CCT interactome was further examined using genomic and proteomic approaches in both yeast and mammalian cells (Dekker et al. 2008; Yam et al. 2008). The list of proteins associated with CCT continues to expand, with current predictions that approximately 10–15% of newly synthesized proteins interact with CCT. Besides cytoskeletal protein components, the CCT interaction network includes proteins involved in many essential cellular processes including cell cycle, cell division, DNA maintenance, replication, repair and recombination, metabolism, transcription and translation, RNA processing, cellular trafficking, signal transduction and interestingly several viral proteins (Fig. 2.2, Supplementary Table 2.1). The importance of CCT to maintain cellular proteostasis is implied in the diversity of its substrates and their function. As shown in Fig. 2.2, CCT is involved to a similar extent in the folding of proteins of major cellular processes with no clear preference for one process. A characteristic of CCT clients is that most of them can be handled only by the chaperonin (Kerner et al. 2005) and at least 40% of substrates consist of essential genes, most of which are conserved among eukaryotes (Yam et al. 2008).

Since substrates of CCT are dissimilar both in structure and function, a sequence or structural motif of recognition in the substrate or the binding site of CCT have not been established to date. Collectively, it indicates a possible lower specificity of the chaperonin. However, analysis of the interactome revealed certain analogies between the substrates. Statistically, CCT has higher affinity for substrates with enrichment in beta sheets in their secondary structure and/or lower content of alpha helices (Yam et al. 2008). Additionally, increased number of hydrophobic residues is observed in the segments of 60-residue polypeptide chain (Yam et al. 2008) and such characteristic contributes to the sequence-based molecular recognition of the substrate (Feldman et al. 2003; Rommelaere et al. 1999). Most of the CCT substrates are in the 40–75 kDa molecular weight size range, which is consistent with the dimensions of the folding cavity of the chaperonin (Ditzel et al. 1998). Approximately 20% of substrates exceed a molecular weight of 75 kDa (Yam et al. 2008), for example myosin heavy chain (223 kDa) (Srikakulam and Winkelmann 1999), which cannot be confined into the chamber and most likely threads through the complex (Rüßmann et al. 2012).

One structural motif that does appear frequently among CCT-associated substrates is the WD40 domain – a short sequence of ~40 amino acids terminated with tryptophan and aspartic

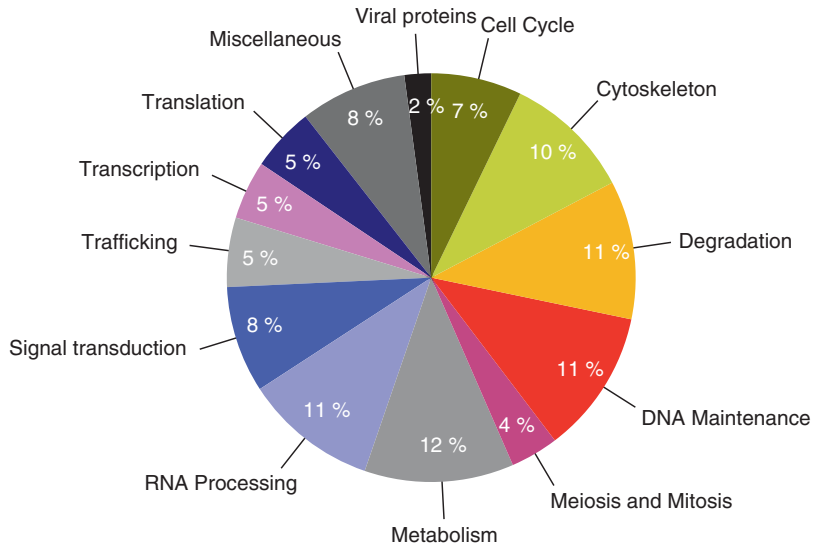

Fig. 2.2 Categorized distribution of proteins identified as part of the CCT interactome. A total of 239 substrates of CCT were classified according to their biological function in the cell, with the addition of a viral protein category. The percentage of each group is indicated within their representative sectors. Denoted categories correspond to groups indicated in Supplementary Table 2.1. Assignments were made on the basis of available scientific reports. Miscellaneous group represents proteins with uncertain/unassigned cellular function

acid dipeptide, whose 4–16 times repetition forms a solenoid beta-propeller blade structure. Several CCT interacting proteins were found to contain a functional WD40 domain (Camasses et al. 2003; Freund et al. 2014; Gavin et al. 2002; Ho et al. 2002; Horwich et al. 2007; Yam et al. 2008). For example, Cdc20 contains 7 WD40 repeats, the central region of which is involved in the interaction with CCT (Camasses et al. 2003).

Interestingly, a vast number of chaperonin clients consist of subunits of large multiprotein complexes, and CCT-mediated folding is crucial for their proper assembly and function (Camasses et al. 2003; Dekker et al. 2008; Feldman et al. 1999; Spiess et al. 2004). It is well established that CCT is essential for the integrity and proper formation of cytoskeleton, by folding and assembly of actin and tubulin (Gao et al. 1992; Hartl 1996; Muñoz et al. 2011; Yaffe et al. 1992). But also actin-related proteins, actin depolarizing factor, myosin or cofilin (Supplementary Table 2.1) (Melki et al. 1997; Melki and Cowan 1994; Srikakulam and Winkelmann 1999; Yaffe et al. 1992) require CCT for proper folding. Another example of substrates fundamental for proper cellular functioning is the cell cycle regulators Cdc20 and Cdh1. CCT assistance is required for the association of Cdc20 or Cdh1 with APC/C (anaphase-promoting complex/cyclosome) complex in the anaphase of cell cycle (Camasses et al. 2003). Furthermore, CCT interacts physically with all the components of the septin complex and is essential for its proper assembly. The importance of this interaction is indicated by the disappearance of the complex in cells mutated with non-functional CCT (Dekker et al. 2008).

How CCT recognizes its substrates remains an open question. A previously proposed hypothesis suggested that the helical protrusions (Fig. 2.1b) of the apical domains are involved in substrate recognition, especially regarding their hydrophobic character and flexibility. However, it was shown that CCT is still capable of binding the substrate after their deletion (Iizuka et al. 2004). Another hypothesis suggests that in homology to the bacterial precursor GroEL, structural

Supplementary Table 2.1 Table represents a gathered list of the proteins associated with CCT, categorized by their cellular function

Human gene	Yeast gene	Literature report
Cell cycle		
CCAR1		Huang et al. (2012)
CCNB (Cyclin B)	CLB2	Melki et al. (1997)
CCNE (Cyclin E)	**CLB5**	Won et al. (1998)
CDC20	**CDC20**	Camasses et al. (2003), Ho et al. (2002), Kaisari et al. (2017), and Yam et al. (2008)
–	**CDC15**	Yam et al. (2008)
CDK2	**CDK1/CDC28**	Ho et al. (2002) and Yam et al. (2008)
CSNK2A1	**CKA1**	Yam et al. (2008)
	DBF20	Ho et al. (2002) and Yam et al. (2008)
FZR1 (CDH1)	**CDH1**	Camasses et al. (2003) and Ho et al. (2002)
	HRT1	Ho et al. (2002) and Yam et al. (2008)
NDC80	**NDC80**	Dekker et al. (2008)
PLK1		Liu et al. (2005)
PPP2R2A	**CDC55**	Ho et al. (2002) and Yam et al. (2008)
	RSR1	Dekker et al. (2008)
SEPT2	**CDC12** **CDC3** **CDC11**	Dekker et al. (2008)
SEPT7	**CDC10**	Dekker et al. (2008), Ho et al. (2002), and Yam et al. (2008)
SMC3	**SMC3**	Gavin et al. (2002)
	VPS64	Ho et al. (2002) and Yam et al. (2008)
Cytoskeleton		
ACTA1, ACTB	**ACT1**	Dekker et al. (2008), Melki et al. (1993), and Yam et al. (2008)
ACTR1A (centractin)	**ARP1**	Yam et al. (2008) and Melki et al. (1993)
ACTR2 (actin-related protein2)	**ARP2**	Dekker et al. (2008), Gavin et al. (2002), Ho et al. (2002), Melki et al. (1993), and Yam et al. (2008)
ADF1/DSTN	–	Melki et al. (1997) and Yam et al. (2008)
	BCH1	Dekker et al. (2008)
	BFA1	Ho et al. (2002) and Yam et al. (2008)
CFL1 (cofilin)	COF1	Melki et al. (1997) and Yam et al. (2008)
DYNC2H1	**DYN1**	Dekker et al. (2008)
	DYN2	Gavin et al. (2002)
FSCN1		Huang et al. (2012)
KIF13A (kinesin family member 13a)	**KIP3**	Yam et al. (2008)
KIFC3	**KAR3**	Yam et al. (2008)
MYH1 (heavy chain myosin)	MYO1	Srikakulam and Winkelmann (1999)
MYH4	**MYO4**	Dekker et al. (2008)
Myosin II	CIN8	Srikakulam and Winkelmann (1999)
	SEY1	Dekker et al. (2008)
	SHS1	Dekker et al. (2008)
	SLA2	Dekker et al. (2008)
	SPC34	Dekker et al. (2008)
TPI1	**TPI1**	Huang et al. (2012) and Yam et al. (2008)

(continued)

Supplementary Table 2.1 (continued)

Human gene	Yeast gene	Literature report
TUBA (α-tubulin)	**TUB1**	Huang et al. (2012), Yaffe et al. (1992), and Yam et al. (2008)
TUBB (β-tubulin)	**TUB2**	Dekker et al. (2008), Munoz et al. (2011), Yaffe et al. (1992), and Yam et al. (2008)
TUBG (γ-tubulin)		Melki et al. (1993)
VIM	–	Yam et al. (2008)
Degradation		
BTRC	–	Yam et al. (2008)
CAPNS1		Yam et al. (2008)
	DIA2	Ho et al. (2002) and Yam et al. (2008)
FBXO4	–	Yam et al. (2008)
FBXL3	–	Yam et al. (2008)
FBXL5	–	Yam et al. (2008)
FBXW2	–	Yam et al. (2008)
FBXW4	–	Yam et al. (2008)
FBXW5	–	Yam et al. (2008)
FBXW8	MET30	Litterman et al. (2011) and Yam et al. (2008)
FBXW9	–	Yam et al. (2008)
FBXW10	–	Yam et al. (2008)
FBXW11	–	Yam et al. (2008)
	GRR1	Dekker et al. (2008)
	HRT3	Ho et al. (2002) and Yam et al. (2008)
PNP	**PNP1**	Yam et al. (2008)
	PRE1	Ho et al. (2002)
PSMA5 (proteasome subunit α t5)	**PUP2**	Yam et al. (2008)
PSMB3	**PUP3**	Yam et al. (2008)
PSMC4	**RPT3**	Ho et al. (2002) and Yam et al. (2008)
	SAN1	Ho et al. (2002) and Yam et al. (2008)
TRIM32		Yam et al. (2008)
UBA1	**UBA1**	Yam et al. (2008)
UCHL3	**YUH1**	Yam et al. (2008)
USP11	UBP12	Yam et al. (2008)
VHL	–	Feldman et al. (1999) and Joachimiak et al. (2014)
DNA maintenance, replication, recombination and repair		
ATR	**RAD3**	Ho et al. (2002) and Yam et al. (2008)
CDC45	**CDC45**	Yam et al. (2008)
	CMR1	Gallina et al. (2015), Ho et al. (2002), and Yam et al. (2008)
HDAC3	RPD3	Guenther et al. (2002) and Yam et al. (2008)
	HOS2	Dekker et al. (2008), Pijnappel et al. (2001), and Yam et al. (2008)
KAT2A	**GCN5**	Ho et al. (2002)
MEAF6	**EAF5**	Dekker et al. (2008)
MGMT	MGT1	Ho et al. (2002) and Yam et al. (2008)
MMS19	**MET18**	Ho et al. (2002) and Yam et al. (2008)
	MTC5	Ho et al. (2002) and Yam et al. (2008)
MUS81	**MUS81**	Ho et al. (2002) and Yam et al. (2008)
PAPD7	**PAP2 (TRF4)**	Ho et al. (2002) and Yam et al. (2008)

(continued)

Supplementary Table 2.1 (continued)

Human gene	Yeast gene	Literature report
PIF1	**PIF1**	Dekker et al. (2008)
POLK	**POL4**	Ho et al. (2002) and Yam et al. (2008)
PSF3	**PSF3**	Dekker et al. (2008)
RAD17	**RAD24**	Ho et al. (2002) and Yam et al. (2008)
	RAD28	Ho et al. (2002) and Yam et al. (2008)
RAD52	**RAD59**	Ho et al. (2002) and Yam et al. (2008)
	RAD55	Ho et al. (2002) and Yam et al. (2008)
RECQL	**SGS1**	Yam et al. (2008)
RFC2	**RFC2**	Ho et al. (2002) and Yam et al. (2008)
RFC3	**RFC3**	Ho et al. (2002) and Yam et al. (2008)
RFC4	**RFC4**	Ho et al. (2002) and Yam et al. (2008)
RPA2	**RFA2**	Ho et al. (2002) and Yam et al. (2008)
	SET3	Yam et al. (2008)
TCAB1		Freund et al. (2014)
	YKU80	Ho et al. (2002) and Yam et al. (2008)
Meiosis and mitosis		
ANAPC13	**SWM1**	Ho et al. (2002) and Yam et al. (2008)
DMC1	**DMC1**	Ho et al. (2002) and Yam et al. (2008)
	IME2	Ho et al. (2002) and Yam et al. (2008)
	PPH22	Ho et al. (2002) and Yam et al. (2008)
PTPA	**PPH21**	Ho et al. (2002) and Yam et al. (2008)
	RLF2	Ho et al. (2002) and Yam et al. (2008)
TP53 (p53)		Trinidad et al. (2013)
TP53RK	**BUD32**	Ho et al. (2002) and Yam et al. (2008)
	WTM1	Ho et al. (2002) and Yam et al. (2008)
Metabolism		
	ACC1	Gavin et al. (2002)
AFMID	**BNA7**	Dekker et al. (2008)
ALDH6A1	**ALD4**	Yam et al. (2008)
ALDOA/C		Huang et al. (2012)
	ARO1	Gavin et al. (2002)
ATP5B		Huang et al. (2012)
	CCC1	Dekker et al. (2008)
	CIR2	Dekker et al. (2008)
	CIT2	Yam et al. (2008)
COX15	**COX15**	Dekker et al. (2008)
	CSH1	Dekker et al. (2008)
CYP1B1		Yam et al. (2008)
DPH1	**DPH1**	Dekker et al. (2008)
ENO1	**ENO1**	Yam et al. (2008)
	FAS2	Yam et al. (2008)
GAPDH	TDH1	Yam et al. (2008)
	GID7	Ho et al. (2002) and Yam et al. (2008)
HMGCS1	**CAB3**	Dekker et al. (2008)
LDHA		Huang et al. (2012)
PDHX	**PDX1**	Dekker et al. (2008)
PFKFB2	**PFK2**	Ho et al. (2002) and Yam et al. (2008)
	PPH3	Ho et al. (2002) and Yam et al. (2008)
PRDX1		Huang et al. (2012)

(continued)

Supplementary Table 2.1 (continued)

Human gene	Yeast gene	Literature report
SORT1	**VID27**	Dekker et al. (2008)
	THI3	Gavin et al. (2002)
VDAC 1/2		Huang et al. (2012)
XYLT1		Huang et al. (2012)
	YCF1	Dekker et al. (2008)
RNA processing		
BOP1	**ERB1**	Ho et al. (2002) and Yam et al. (2008)
CCDC94	**YJU2**	Ho et al. (2002) and Yam et al. (2008)
CNOT6	**CCR4**	Gavin et al. (2002)
CPSF6	**PFS2**	Ho et al. (2002) and Yam et al. (2008)
CSTF3		Huang et al. (2012)
CYFIP1		Huang et al. (2012)
DCAF13	**SOF1**	Ho et al. (2002) and Yam et al. (2008)
EDC3	**EDC3**	Dekker et al. (2008)
EEF1 A1/2 B1		Huang et al. (2012)
EEF2		Huang et al. (2012)
EFTUD2		Huang et al. (2012)
	ENP2	Dekker et al. (2008)
HNRNP C/D		Huang et al. (2012)
LSM2	**LSM2**	Ho et al. (2002) and Yam et al. (2008)
NOP2	**NOP2**	Ho et al. (2002) and Yam et al. (2008)
NOP56	**NOP56/SIK1**	Yam et al. (2008)
NPM1		Yam et al. (2008)
	PRP46	Ho et al. (2002) and Yam et al. (2008)
RPL23A	**RPL23A**	Dekker et al. (2008)
RPL36A	**RPL36A**	Dekker et al. (2008)
RPS16	RPS16A	Huang et al. (2012)
SNRNP200		Huang et al. (2012)
UTP11	**UTP7**	Ho et al. (2002) and Yam et al. (2008)
U2AF2	**HSH155**	Dekker et al. (2008)
XRN2	RAT1	Yam et al. (2008)
Signal transduction		
AML1-ETO (oncogenic protein fusion)	–	Roh et al. (2016)
	COS111	Yam et al. (2008)
	EAP1	Dekker et al. (2008)
	FAR1	Ho et al. (2002) and Yam et al. (2008)
GNAT1	**GPA1**	Farr et al. (1997) and Yam et al. (2008)
GNB4	ASC1	Wells et al. (2006) and Yam et al. (2008)
	KSS1	Ho et al. (2002) and Yam et al. (2008)
	LEM3	Ho et al. (2002) and Yam et al. (2008)
LOX		Bakthavatsalam et al. (2014)
NEDD1		am et al. (2008)
PDCL	**PLP1** **PLP2**	Dekker et al. (2008), Gao et al. (2013), Howlett et al. (2009), and Martin-Benito et al. (2004)
	SMK1	Ho et al. (2002) and Yam et al. (2008)
	SIP2	Ho et al. (2002) and Yam et al. (2008)

(continued)

Supplementary Table 2.1 (continued)

Human gene	Yeast gene	Literature report
	SIT4	Gavin et al. (2002), Ho et al. (2002) and Yam et al. (2008)
SPHK1	**SK1**	Zebol et al. (2009)
STAT3		Kasembeli et al. (2014)
	STE4	Ho et al. (2002)
TAOK1	**PSK2**	Ho et al. (2002) and Yam et al. (2008)
	TAP42	Gavin et al. (2002)
	TEM1	Ho et al. (2002), and Yam et al. (2008)
Trafficking		
	APM1	Ho et al. (2002)
AP3M1	**APM3** **APM4**	Yam et al. (2008)
ARFGEF2	**SEC7**	Yam et al. (2008)
DOPEY1/ DOPEY2	**DOP1**	Dekker et al. (2008)
HSPD1	**HSP60**	Dekker et al. (2008)
	MNN1	Dekker et al. (2008)
MLST8	**LST8**	Ho et al. (2002) and Yam et al. (2008)
MTOR	**KOG1**	Ho et al. (2002) and Yam et al. (2008)
NFX1/TAP	MEX67	Yam et al. (2008)
PEX7	**PEX7**	Ho et al. (2002) and Yam et al. (2008)
	PEX25	Dekker et al. (2008)
PPIA		Huang et al. (2012)
	SEC27	Yam et al. (2008) and Ho et al. (2002)
Transcription		
	CAF4	Ho et al. (2002) and Yam et al. (2008)
MED15		Yam et al. (2008)
POLR1C	**RPC40**	Ho et al. (2002) and Yam et al. (2008)
POLR2G	**RPB7**	Dekker et al. (2008)
POLR3A (DNA-directed RNA polymerase III subunit RPC1)	RPO31	Yam et al. (2008)
	RTT102	Dekker et al. (2008)
	SWI3	Dekker et al. (2008)
TAF5	**TAF90**	Ho et al. (2002) and Yam et al. (2008)
TSHZ3		Yam et al. (2008)
	WTM2	Ho et al. (2002) and Yam et al. (2008)
Translation		
AARS	ALA1	Yam et al. (2008)
Aminoacyl-tRNA synthetase class-II protein		Yam et al. (2008)
eEF2	EFT2	Dekker et al. (2008) and Yam et al. (2008)
EIF3A	RPG1	Dekker et al. (2008)
EIF3B/H	**GCN3**	Gavin et al. (2002) and Roobol et al. (2014)
EIF3I	TIF34	Roobol et al. (2014) and Yam et al. (2008)
FARSA	**FRS2**	Dekker et al. (2008)
LARS	CDC60	Yam et al. (2008)
RARS	YDR341C	Yam et al. (2008)
RPLP0	**RPP0**	Ho et al. (2002) and Yam et al. (2008)
	UTP13	Ho et al. (2002) and Yam et al. (2008)
	VAR1	Dekker et al. (2008)

(continued)

Supplementary Table 2.1 (continued)

Human gene	Yeast gene	Literature report
VARS		Huang et al. (2012)
Miscellaneous/unknown		
AFAP1		Yam et al. (2008)
AMOTL2		Yam et al. (2008)
API5		Huang et al. (2012)
BRD1	**NTO1**	Yam et al. (2008)
	ASR1	Ho et al. (2002) and am et al. (2008)
CTNNA1		Yam et al. (2008)
	CUZ1	Dekker et al. (2008)
	FAP1	Dekker et al. (2008)
FKBP9	FPR2	Yam et al. (2008)
HNRNPM		Yam et al. (2008)
	INP52	Ho et al. (2002) and Yam et al. (2008)
	IRC10	Dekker et al. (2008)
	MOH1	Ho et al. (2002) and Yam et al. (2008)
PARK7 (DJ-1)		Yam et al. (2008)
PWP1	**PWP1**	Ho et al. (2002) and Yam et al. (2008)
SFPQ		Huang et al. (2012)
TK1		Yam et al. (2008)
	YBR209W	Ho et al. (2002)
	YGL081W	Ho et al. (2002) and Yam et al. (2008)
	YRR1	Dekker et al. (2008)
Viral proteins		
EBNA-3 (Epstein-Barr virus-encoded nuclear protein)		Kashuba et al. (1999)
Hepatitis B virus capsid proteins		Lingappa et al. (1994)
NS5B (Hepatitis C)		Inoue et al. (2011)
Nucleo- and phoshoproteins of RABV		Zhang et al. (2013)
p4 of M-PMV		Hong et al. (2001)
p6 of HIV-1		Hong et al. (2001), Jager et al. (2011), and Joachimiak et al. (2014)

Name of the human gene, yeast gene and literature report are indicated in three columns. Reported interaction is represented by the associated gene name. Last category represents a list of the viral proteins known to be interacting with CCT

elements of the apical domain, namely two helices in the distal region of the domain are involved in substrate binding and recognition (Buckle et al. 1997). Recently, this hypothesis was experimentally supported by a study of the most important regions required for the interaction between the apical domain of CCT and a substrate. A shallow groove formed by α-helix 11 (Fig. 2.1b) and a flexible proximal loop were identified by NMR analysis as the substrate binding regions (Joachimiak et al. 2014). Different paralogs of CCT demonstrate diversity in the residue composition and mixed chemical properties in this area, which seems to be the feature that enables CCT to recognize a large variety of protein clients (Lopez et al. 2015).

2.4 Conformational Changes and Nucleotide Hydrolysis

In the course of evolution, group I and group II chaperonins have adopted different mechanisms to close the binding cavities in which the substrates are encapsulated. In the bacterial chaperonin system, GroES functions as a detachable lid to close the binding chamber of GroEL (Langer et al. 1992). However, there is no co-chaperonin

corresponding to GroES in eukaryotes to help with the closure of the complex. The built-in-lid structure formed by apical domains is specific to group II chaperonins, such as CCT (Kim et al. 2013).

The apical domain initiates substrate recognition (Kim et al. 1994; Spiess et al. 2006). The open-conformational structure of bovine CCT in complex with tubulin illustrated how the substrate interacts with loops of the apical and equatorial domains (Muñoz et al. 2011). Unexpectedly, in addition to its lid function, the apical protrusions were also found to work as an allosteric regulator to modulate the conformational change in an ATP-dependent manner (Muñoz et al. 2011). Both the entire CCT and the lidless CCT could bind unfolded actin, but only the lid containing version folded the substrate successfully, suggesting that the built-in-lid is essential for a productive folding process (Reissmann et al. 2007). The chaperonin was not capable of facilitating substrate folding when the lid was removed. To detect the function of the apical domains in conformational changes, Iizuka et al. constructed three mutants of the hyperthermophilic archaeal chaperonin by deleting helical protrusions in apical domains. Protease sensitivity assays demonstrated that the addition of ATP induced conformational changes that protect the protein from proteolysis in wild type CCT but not in the mutants, suggesting that the addition of nucleotides induced closure of the cavity. This result was confirmed by small angle x-ray scattering experiments as well (Iizuka et al. 2004). Taken together, these experiments supported the importance of the apical domains in the allosteric regulation of the chaperonin.

The hydrolysis of ATP is essential to induce the conformational changes in both intra- and inter-rings and trigger the closure of the folding chamber by the assembly of the apical domains. In the absence of nucleotides, CCT remains in an open conformation to ensure access to the substrate binding sites in the apical domains. In the presence of ATPγS and AMP-PNP, non-hydrolysable ATP analogues, CCT underwent a conformational change but still remained in the open conformation, as observed in accessibility experiments using proteases. This demonstrates that binding of the nucleotide in the equatorial domain induces conformational changes but is not sufficient to close the barrel (Meyer et al. 2003). Moreover, after ADP was added, CCT was degraded by proteases, which indicated that the complex remained in the open configuration and the substrate binding sites were still available. Accordingly, the conformational changes of CCT are ATP hydrolysis-dependent (Meyer et al. 2003; Szpikowska et al. 1998). The conformational change induced in the apical domains by the hydrolysis of ATP on the equatorial domains isolates the substrate from the cellular environment closing the cavity and starting the folding process. This lid closure mechanism is completely different to GroEL, which needs GroES to cap the binding chamber when ATP binds to the *cis* side of the equatorial domain of GroEL (Rye et al. 1997).

The equatorial domains containing the nucleotide binding sites are relatively stationary. CCT utilizes a complex non-concerted sequential action to close the central chamber. In a study of the *Thermococcus* chaperonin, the mutated subunits were designed in an ordered manner with impaired ability of ATP hydrolysis or conformational change. The effect of mutants on conformational change ability correlated with the number and order in the ring (Kanzaki et al. 2008). Recently, it has been reported that the subunits have different ATP binding affinity and the four low-affinity subunits are not necessary for CCT to perform its function *in vivo*. This has been previously observed in the CCT-tubulin complex where the ATPγS molecule was observed in four subunits only (Muñoz et al. 2011). Taking the order of subunits into consideration, the high-affinity subunits and the low-affinity subunits are segregated in the relative symmetric rings resulting in a remarkable asymmetry in the ATP powered folding cycle (Reissmann et al. 2012). Moreover, the non-concerted mechanism was confirmed by the crys-

tal structure of CCT with tubulin. Each subunit possessed a different conformation illustrating an asymmetric pattern (Muñoz et al. 2011; Yébenes et al. 2011), which is reflected in the position of the ATP binding sites when compared with the fully closed conformation. Therefore, when CCT is in its open conformation, the non-native substrate is recognized and captured by the apical domains and ATP binds to the equatorial domain. Coupled with the hydrolysis of ATP, CCT experiences a switch from open to closed conformation and confines the substrate into the binding chamber. If the substrate is not folded properly, it can be released and additional attempts of the folding cycle will follow (Farr et al. 1997). The substrate biding sites on apical domains become accessible again as soon as CCT recovers its open conformation and the previous protein substrate is released. Thus, the complex is ready for the next protein client. The protein folding cycle is illustrated in Fig. 2.3.

2.5 CCT and Cancer

Protein biogenesis is an essential process for cell function and viability and it is based on an extremely balanced equilibrium between protein synthesis and modification, degradation and assisted folding. In the context of cancer, where some cell functions are extremely disrupted, protein homeostasis could be hijacked, as other pro-growth cellular pathways, and the action of CCT provides improved conditions for proliferation.

Different studies have shown that CCT expression levels in cancer cell lines are higher than in normal cells (Boudiaf-Benmammar et al. 2013). Specifically, recent experimental data supported by analysis of clinical data compiled by initiatives like The Cancer Genome Atlas (Tomczak et al. 2015) showed a link between certain types of cancer and over-expression of specific CCT subunits. In this regard, it has been shown that CCTβ and CCTα frequently present expression

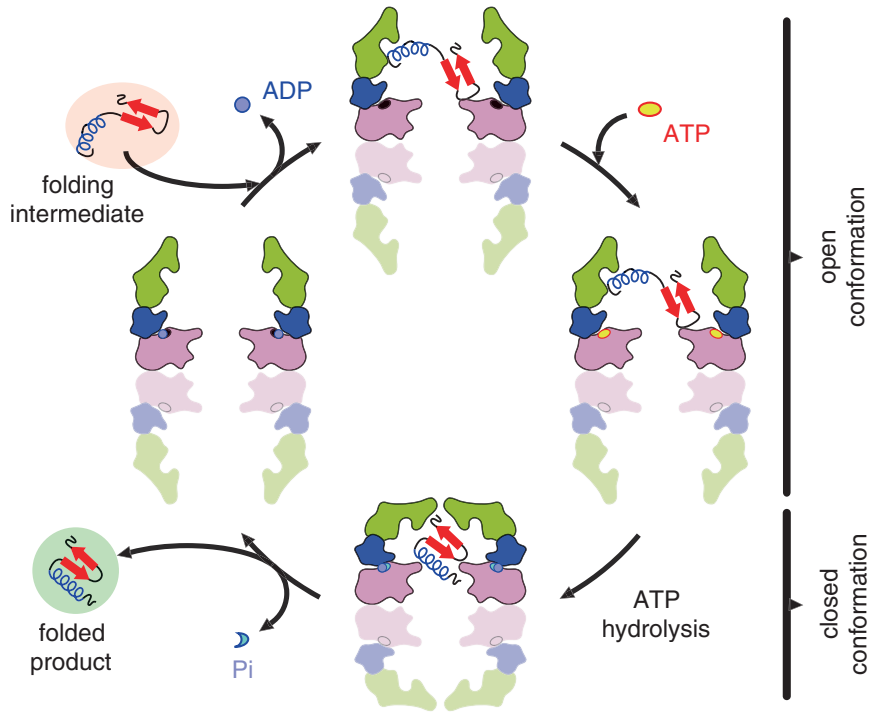

Fig. 2.3 CCT ATP-dependent folding cycle. Hypothetical model for the folding of a CTT substrate, depicted as a folding intermediate that needs some assistance to reach its native structure. The ADP∗Pi hydrolysis steep represents the active conformational change that closes the chamber and promotes the folding process. The activity of only one of the rings is shown, since both rings can be in different open/closed conformations

alterations in breast cancer. They are necessary for breast cancer cell proliferation and colony formation, and they are also associated with poor overall survival of breast cancer patients (Guest et al. 2015). High levels of CCTγ expression have been also related with gastric cancer growth and survival (Li et al. 2017). This subunit was also linked with hepatocellular carcinoma, colon cancer and cholangiocarcinoma ((Zhang et al. 2016); Yokota et al. 2001; Shi et al. 2013). Furthermore, the inhibition of CCTζ-1 expression by siRNA produced growth arrest in colorectal carcinoma cells (Qian-Lin et al. 2010). It is not clear how the over-expression of individual CCT subunits can help cancer cells, but it has been indicated that certain CCT subunits are able to constitute functional, at least *in vitro*, homo-oligomers (Sergeeva et al. 2013). Furthermore, CCT plays an indirect role in oncogenesis, by folding of cancer-related proteins, such as Von Hippel-Lindau (VHL), p53, and signal transducer and activator of transcription 3 (STAT3).

2.6 CCT as Key Regulator of Cell Cycle

The importance of CCT as a therapeutic target to fight proliferation is not only related with its role in maintaining proteostasis. This chaperonin is directly implicated in the regulation of processes that are related to cell cycle and tumor progression. Among them are cytoskeleton organization as well as G1/S, G2/M and anaphase transitions though the folding of specific key substrates. CCT controls the folding of cyclin E (Won et al. 1998) and B (Boudiaf-Benmammar et al. 2013; Melki et al. 1997; Melki and Cowan 1994), thus influencing their assembly with Cdk2 and Cdk1 respectively (Fig. 2.4). These complexes control G1/S and G2/M transitions. CCT also regulates the APC/C E3 ubiquitin ligase through folding of its regulatory subunits Cdc20 and Cdh1 (Camasses et al. 2003).

The cyclin E/Cdk2 complex triggers the Retinoblastoma/E2F and phosphorylates p27kip1, labelling it for degradation and inducing the expression of cyclin A, allowing progression into S-Phase (Blow and Gillespie 2008). It also seems to be involved in the centrosome cycle. Mouse models bearing a mutant cyclin E that cannot be targeted for degradation turn it into an oncogene (Loeb et al. 2005). Cyclin B, also known as the mitotic cyclin, raises its levels during cell cycle till the end of mitosis. Its assembly into the CyclinB/Cdk1 complex is necessary for progression into M-phase. Cyclin B plays an integral role in many types of cancer and its levels are often de-regulated in tumors. The silencing of this gene increases susceptibility to taxol and leads to growth arrest *in vivo* (Yuan et al. 2006). The APC/C controls key events in mitosis, such as sister chromatid separation and subsequent inactivation of Cdk1, targeting securin and cyclins for degradation (Irniger et al. 1995; Sudakin et al. 1995). The APC/C (Barford 2011) is only fully active once it assembles with Cdc20, Cdh1, or related activators, resulting in distinct complexes. Since Cdc20 and Cdh1 can bind to APC/C substrates, they may activate ubiquitination reactions by recruiting different substrates to the APC/C (Peters 2006). In mitosis, binding to Cdc20 activates APC/C, and this event is dependent on high Cdk1 activity. Cyclin B degradation begins in metaphase and continues while sister chromatids separate in anaphase. However, to accomplish this process securin must be also degraded allowing separase to digest the cohesin ring. This mechanism requires the assembly of Cdc20 into the APC/C and this association is catalyzed by CCT, which is essential to turn Cdc20 into a folded polypeptide. Recently it has been shown that CCT is involved in the active disassembly of mitotic checkpoint complexes by the release of Cdc20 (Kaisari et al. 2017), meaning that the role of CCT can be extended even beyond its ordinary protein folding function. The action of CCT has also been shown to be crucial in vivo for sister chromatid separation and exit from mitosis, even in cells lacking checkpoint pathways (Camasses et al. 2003). Mouse models and functional experiments have shown the relevance of cyclins E and B in proliferation (Loeb et al. 2005; Yuan et al. 2006). In the case of Cdc20, which is essential for anaphase onset in vivo in embryonic or adult cells (including

progenitor/stem cells), it has been recently shown that its ablation results in efficient regression of aggressive tumors, whereas current mitotic drugs display limited effects (Manchado et al. 2010).

Consequently, CCT is a key complex, it links G1/S, G2/M, metaphase-anaphase transitions and cytoskeleton organization, and modulates the cancer-related proteins, suggesting that this cellular hub joining cell cycle progression, microtubule growth, and oncogenesis, could be a key point where direct inhibition, or its combination with other drugs may be an effective cancer treatment.

2.7 Is CCT a Possible Antiproliferation Target?

The search for new antitumoral targets is one of the most active fields in cancer research. The identification of potential targets must be based on evidence of critical biological function in the clinical condition to be addressed and focused on evidence that the function of the target can be productively manipulated. The important role of this cellular function in cell proliferation has already been targeted. Molecular chaperones such as the heat shock protein 90 (HSP90) (an evolutionarily conserved molecular chaperone that participates in stabilizing and activating more than 200 proteins) constitute an active area of research. Many of its substrates are essential for constitutive cell signaling and adaptive responses to stress. Cancer cells use the HSP90 chaperone machinery to protect an array of mutated and over-expressed oncoproteins from misfolding and degradation. Therefore, HSP90 is recognized as a crucial facilitator of oncogene addiction and cancer cell survival. Owing to extensive efforts in rational drug design and discovery, HSP90 inhibitors are currently undergoing clinical evaluation in cancer patients, and some of them have entered the clinic in the last years. However, HSP90 does not display specificity for a subset of substrates involved in mitosis or cytoskeleton organization like CCT, suggesting that the latter may provide higher specificity.

Microtubule inhibitors such as taxanes and the vinca alkaloids represent one of the most important classes of cancer drugs. How these drugs cause cell death remains unclear, but induction of mitotic arrest appears to be a key aspect of the mechanism. It has been proposed that by perturbing the mitotic spindle, these drugs activate the spindle assembly checkpoint, which delays mitotic exit by inhibiting the ubiquitin ligase activity of the APC/C. However, recent analysis challenged this view (Komlodi-Pasztor et al. 2011; Mitchison 2012), which is contradictory with the fact that these compounds act in tumors whose proliferation rates are low, both in absolute terms and relative to a highly proliferative tissue such as bone marrow. This suggest that drugs such as paclitaxel may work beyond the effects described in mitosis, perhaps by improved retention in tumor cells, killing of quiescent cells, targeting of non-cancer cells in the tumor or by triggering other effects after targeting the mitotic cells. The fact that CCT provides a link between mitotic regulation and cytoskeleton organization indicates that compounds targeting this molecule could also provide a dual action that may be improved in combination with microtubule drugs. Thus CCT, which is a cellular hub joining both cell cycle progression and microtubule biogenesis, could be a key point where direct inhibition in combination with other drugs may act synergistically.

Several different strategies have been developed so far to target CCT for therapeutic purposes. For example, it has been shown that the chemical drug I-Trp, that targets the contact interface between β-tubulin and CCT-β, can selectively kill several cancer cell lines over-expressing CCT-β (Liu et al. 2017). Also, it has been demonstrated that a small peptide, called CT20p, was cytotoxic for small cell lung cancer cell lines with up-regulated CCTβ (Carr et al. 2017). The main drawback for these strategies is that CCT is an essential gene and modifying its activity could affect important cellular processes in healthy cells (Figs. 2.2 and 2.4). Further research is needed to find out whether downregulating CCT function will prove to be an effective anti-cancer strategy.

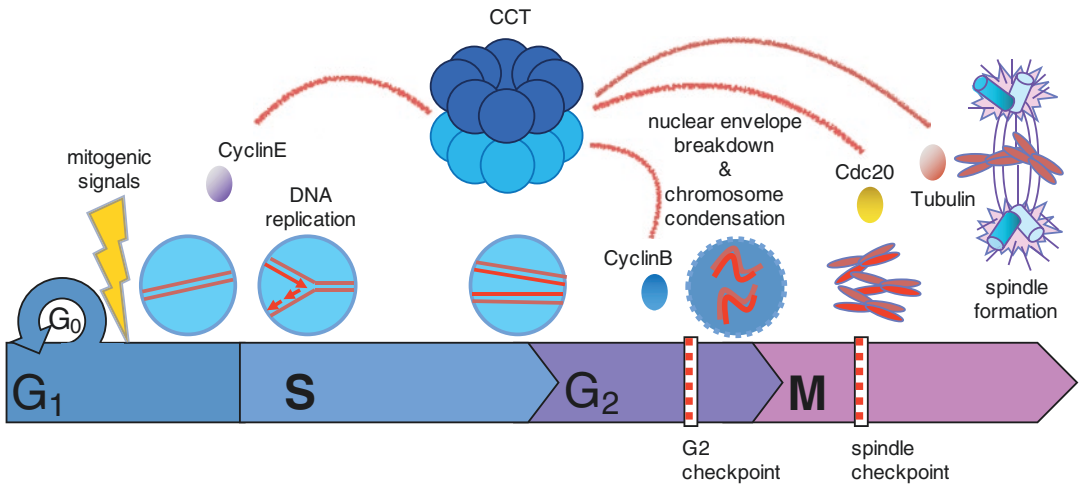

Fig. 2.4 Role of CCT as a central hub in the cell cycle by controlling the folding of key substrates, like CyclinE, Cyclin B, Cdc20 and tubuline. Cyclin E and B assemble with Cdk2 and Cdk1 respectively to modulate G1/S and G2/M transitions. Cdc20 activates APC/C to initiate chromatid separation and entrance into anaphase. The cytoskeleton protein tubulin is involved in spindle formation

Acknowledgements The Novo Nordisk Foundation Center for Protein Research is supported financially by the Novo Nordisk Foundation (Grant NNF14CC0001). This work was also supported by the cryoEM (Grant NNF0024386) and cryoNET (Grant NNF17SA0030214) grants to GM, Guillermo Montoya is a member of the Integrative Structural Biology Cluster (ISBUC) at the University of Copenhagen.

References

Bakthavatsalam D, Soung RH, Tweardy DJ, Chiu W, Dixon RA, Woodside DG (2014) Chaperonin-containing TCP-1 complex directly binds to the cytoplasmic domain of the LOX-1 receptor. FEBS Lett 588:2133–2140

Barford D (2011) Structural insights into anaphase-promoting complex function and mechanism. Philos Trans R Soc Lond Ser B Biol Sci 366:3605–3624

Blow JJ, Gillespie PJ (2008) Replication licensing and cancer – a fatal entanglement? Nat Rev Cancer 8:799–806

Booth CR, Meyer AS, Cong Y, Topf M, Sali A, Ludtke SJ, Chiu W, Frydman J (2008) Mechanism of lid closure in the eukaryotic chaperonin TRiC/CCT. Nat Struct Mol Biol 15:746–753

Boudiaf-Benmammar C, Cresteil T, Melki R (2013) The cytosolic chaperonin CCT/TRiC and cancer cell proliferation. PLoS One 8:e60895

Buckle AM, Zahn R, Fersht AR (1997) A structural model for GroEL-polypeptide recognition. Proc Natl Acad Sci U S A 94:3571–3575

Camasses A, Bogdanova A, Shevchenko A, Zachariae W (2003) The CCT chaperonin promotes activation of the anaphase-promoting complex through the generation of functional Cdc20. Mol Cell 12:87–100

Carr AC, Khaled AS, Bassiouni R, Flores O, Nierenberg D, Bhatti H, Vishnubhotla P, Manuel JP, Santra S, Khaled AR (2017) Targeting chaperonin containing TCP1 (CCT) as a molecular therapeutic for small cell lung cancer. Oncotarget 8(66):110273–110288

Dekker C, Stirling PC, Mccormack EA, Filmore H, Paul A, Brost RL, Costanzo M, Boone C, Leroux MR, Willison KR (2008) The interaction network of the chaperonin CCT. EMBO J 27:1827–1839

Ditzel L, Löwe J, Stock D, Stetter K-O, Huber H, Huber R, Steinbacher S (1998) Crystal structure of the thermosome, the archaeal chaperonin and homolog of CCT. Cell 93:125–138

Dobson CM, Šali A, Karplus M (1998) Protein folding: a perspective from theory and experiment. Angew Chem Int Ed 37:868–893

Douglas NR, Reissmann S, Zhang J, Chen B, Jakana J, Kumar R, Chiu W, Frydman J (2011) Dual action of ATP hydrolysis couples lid closure to substrate release into the group II chaperonin chamber. Cell 144:240–252

Farr GW, Scharl EC, Schumacher RJ, Sondek S, Horwich AL (1997) Chaperonin-mediated folding in the eukaryotic cytosol proceeds through rounds of release of native and nonnative forms. Cell 89:927–937

Feldman DE, Thulasiraman V, Ferreyra RG, Frydman J (1999) Formation of the VHL-elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. Mol Cell 4:1051–1061

Feldman DE, Spiess C, Howard DE, Frydman J (2003) Tumorigenic mutations in VHL disrupt folding in vivo by interfering with chaperonin binding. Mol Cell 12:1213–1224

Freund A, Zhong FL, Venteicher AS, Meng Z, Veenstra TD, Frydman J, Artandi SE (2014) Proteostatic control of telomerase function through TRiC-mediated folding of TCAB1. Cell 159:1389–1403

Gallina I, Colding C, Henriksen P, Beli P, Nakamura K, Offman J, Mathiasen DP, Silva S, Hoffmann E, Groth A, Choudhary C, Lisby M (2015) Cmr1/WDR76 defines a nuclear genotoxic stress body linking genome integrity and protein quality control. Nat Commun 6:6533

Gao Y, Thomas JO, Chow RL, Lee G-H, Cowan NJ (1992) A cytoplasmic chaperonin that catalyzes β-actin folding. Cell 69:1043–1050

Gao X, Sinha S, Belcastro M, Woodard C, Ramamurthy V, Stoilov P, Sokolov M (2013) Splice isoforms of phosducin-like protein control the expression of heterotrimeric G proteins. J Biol Chem 288:25760–25768

Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415:141–147

Guenther MG, Yu J, Kao GD, Yen TJ, Lazar MA (2002) Assembly of the SMRT-histone deacetylase 3 repression complex requires the TCP-1 ring complex. Genes Dev 16:3130–3135

Guest ST, Kratche ZR, Bollig-Fischer A, Haddad R, Ethier SP (2015) Two members of the TRiC chaperonin complex, CCT2 and TCP1 are essential for survival of breast cancer cells and are linked to driving oncogenes. Exp Cell Res 332(2):223–235

Gupta R (1990) Sequence and structural homology between a mouse T-complex protein TCP-1 and the 'chaperonin' family of bacterial (GroEL, 60–65 kDa heat shock antigen) and eukaryotic proteins. Biochem Int 20:833–841

Gutsche I, Essen L-O, Baumeister W (1999) Group II chaperonins: new TRiC (k) s and turns of a protein folding machine. J Mol Biol 293:295–312

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–580

Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333:330–334

Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutilier K, Yang L, Wolting C, Donaldson I, Schandorff S, Shewnarane J, Vo M, Taggart J, Goudreault M, Muskat B, Alfarano C, Dewar D, Lin Z, Michalickova K, Willems AR, Sassi H, Nielsen PA, Rasmussen KJ, Andersen JR, Johansen LE, Hansen LH, Jespersen H, Podtelejnikov A, Nielsen E, Crawford J, Poulsen V, Sorensen BD, Matthiesen J, Hendrickson RC, Gleeson F, Pawson T, Moran MF, Durocher D, Mann M, Hogue CW, Figeys D, Tyers M (2002) Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. Nature 415:180–183

Hong S, Choi G, Park S, Chung AS, Hunter E, Rhee SS (2001) Type D retrovirus Gag polyprotein interacts with the cytosolic chaperonin TRiC. J Virol 75:2526–2534

Horwich AL, Fenton WA, Chapman E, Farr GW (2007) Two families of chaperonin: physiology and mechanism. Annu Rev Cell Dev Biol 23:115–145

Howlett AC, Gray AJ, Hunter JM, Willardson BM (2009) Role of molecular chaperones in G protein beta5/regulator of G protein signaling dimer assembly and G protein betagamma dimer specificity. J Biol Chem 284:16386–16399

Huang R, Yu M, Li CY, Zhan YQ, Xu WX, Xu F, Ge CH, Li W, Yang XM (2012) New insights into the functions and localization of nuclear CCT protein complex in K562 leukemia cells. Proteomics Clin Appl 6:467–475

Iizuka R, So S, Inobe T, Yoshida T, Zako T, Kuwajima K, Yohda M (2004) Role of the helical protrusion in the conformational change and molecular chaperone activity of the archaeal group II chaperonin. J Biol Chem 279:18834–18839

Inoue Y, Aizaki H, Hara H, Matsuda M, Ando T, Shimoji T, Murakami K, Masaki T, Shoji I, Homma S, Matsuura Y, Miyamura T, Wakita T, Suzuki T (2011) Chaperonin TRiC/CCT participates in replication of hepatitis C virus genome via interaction with the viral NS5B protein. Virology 410:38–47

Irniger S, Piatti S, Michaelis C, Nasmyth K (1995) Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. Cell 81:269–278

Jager S, Cimermancic P, Gulbahce N, Johnson JR, Mcgovern KE, Clarke SC, Shales M, Mercenne G, Pache L, Li K, Hernandez H, Jang GM, Roth SL, Akiva E, Marlett J, Stephens M, D'orso I, Fernandes J, Fahey M, Mahon C, O'donoghue AJ, Todorovic A, Morris JH, Maltby DA, Alber T, Cagney G, Bushman FD, Young JA, Chanda SK, Sundquist WI, Kortemme T, Hernandez RD, Craik CS, Burlingame A, Sali A, Frankel AD, Krogan NJ (2011) Global landscape of HIV-human protein complexes. Nature 481:365–370

Joachimiak LA, Walzthoeni T, Liu CW, Aebersold R, Frydman J (2014) The structural basis of substrate recognition by the eukaryotic chaperonin TRiC/CCT. Cell 159:1042–1055

Kaisari S, Sitry-Shevah D, Miniowitz-Shemtov S, Teichner A, Hershko A (2017) Role of CCT chap-

eronin in the disassembly of mitotic checkpoint complexes. Proc Natl Acad Sci U S A 114:956–961

Kalisman N, Adams CM, Levitt M (2012) Subunit order of eukaryotic TRiC/CCT chaperonin by cross-linking, mass spectrometry, and combinatorial homology modeling. Proc Natl Acad Sci U S A 109:2884–2889

Kanzaki T, Iizuka R, Takahashi K, Maki K, Masuda R, Sahlan M, Yébenes H, Valpuesta JM, Oka T, Furutani M (2008) Sequential action of ATP-dependent subunit conformational change and interaction between helical protrusions in the closure of the built-in lid of group II chaperonins. J Biol Chem 283:34773–34784

Kasembeli M, Lau WC, Roh SH, Eckols TK, Frydman J, Chiu W, Tweardy DJ (2014) Modulation of STAT3 folding and function by TRiC/CCT chaperonin. PLoS Biol 12:e1001844

Kashuba E, Pokrovskaja K, Klein G, Szekely L (1999) Epstein-Barr virus-encoded nuclear protein EBNA-3 interacts with the epsilon-subunit of the T-complex protein 1 chaperonin complex. J Hum Virol 2:33–37

Kerner MJ, Naylor DJ, Ishihama Y, Maier T, Chang H-C, Stines AP, Georgopoulos C, Frishman D, Hayer-Hartl M, Mann M (2005) Proteome-wide analysis of chaperonin-dependent protein folding in Escherichia coli. Cell 122:209–220

Kim S, Willison KR, Horwich AL (1994) Cystosolic chaperonin subunits have a conserved ATPase domain but diverged polypeptide-binding domains. Trends Biochem Sci 19:543–548

Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355

Klumpp M, Baumeister W, Essen L-O (1997) Structure of the substrate binding domain of the thermosome, an archaeal group II chaperonin. Cell 91:263–270

Komlodi-Pasztor E, Sackett D, Wilkerson J, Fojo T (2011) Mitosis is not a key target of microtubule agents in patient tumors. Nat Rev Clin Oncol 8:244–250

Kubota H, Hynes G, Carne A, Ashworth A, Willison K (1994) Identification of six Tcp-1-related genes encoding divergent subunits of the TCP-1-containing chaperonin. Curr Biol 4:89–99

Langer T, Pfeifer G, Martin J, Baumeister W, Hartl F (1992) Chaperonin-mediated protein folding: GroES binds to one end of the GroEL cylinder, which accommodates the protein substrate within its central cavity. EMBO J 11:4757–4765

Leitner A, Joachimiak LA, Bracher A, Mönkemeyer L, Walzthoeni T, Chen B, Pechmann S, Holmes S, Cong Y, Ma B (2012) The molecular architecture of the eukaryotic chaperonin TRiC/CCT. Structure 20:814–825

Lewis VA, Hynes GM, Zheng D, Saibil H, Willison K (1992) T-complex polypeptide-1 is a subunit of a heteromeric particle in the eukaryotic cytosol. Nature 358:249–252

Li LJ, Zhang LS, Han ZJ, He ZY, Chen H, Li YM (2017) Chaperonin containing TCP-1 subunit 3 is critical for gastric cancer growth. Oncotarget 8(67):111470–111481

Lingappa JR, Martin RL, Wong ML, Ganem D, Welch WJ, Lingappa VR (1994) A eukaryotic cytosolic chaperonin is associated with a high molecular weight intermediate in the assembly of hepatitis B virus capsid, a multimeric particle. J Cell Biol 125:99–111

Litterman N, Ikeuchi Y, Gallardo G, O'connell BC, Sowa ME, Gygi SP, Harper JW, Bonni A (2011) An OBSL1-Cul7Fbxw8 ubiquitin ligase signaling mechanism regulates Golgi morphology and dendrite patterning. PLoS Biol 9:e1001060

Liu X, Lin CY, Lei M, Yan S, Zhou T, Erikson RL (2005) CCT chaperonin complex is required for the biogenesis of functional Plk1. Mol Cell Biol 25:4993–5010

Liu YJ, Kumar V, Lin YF, Liang PH (2017) Disrupting CCT-β: β-tubulin selectively kills CCT-β overexpressed cancer cells through MAPKs activation. Cell Death Dis 8(9):e3052

Llorca O, Smyth MG, Marco S, Carrascosa JL, Willison KR, Valpuesta JM (1998) ATP binding induces large conformational changes in the apical and equatorial domains of the eukaryotic chaperonin containing TCP-1 complex. J Biol Chem 273:10091–10094

Loeb KR, Kostner H, Firpo E, Norwood T, Tsuchiya KD, Clurman BE, Roberts JM (2005) A mouse model for cyclin E-dependent genetic instability and tumorigenesis. Cancer Cell 8:35–47

Lopez T, Dalton K, Frydman J (2015) The mechanism and function of group II chaperonins. J Mol Biol 427:2919–2930

Manchado E, Guillamot M, DE Carcer G, Eguren M, Trickey M, Garcia-Higuera I, Moreno S, Yamano H, Canamero M, Malumbres M (2010) Targeting mitotic exit leads to tumor regression in vivo: modulation by Cdk1, Mastl, and the PP2A/B55alpha, delta phosphatase. Cancer Cell 18:641–654

Martin-Benito J, Bertrand S, Hu T, Ludtke PJ, Mclaughlin JN, Willardson BM, Carrascosa JL, Valpuesta JM (2004) Structure of the complex between the cytosolic chaperonin CCT and phosducin-like protein. Proc Natl Acad Sci U S A 101:17410–17415

Melki R, Cowan NJ (1994) Facilitated folding of actins and tubulins occurs via a nucleotide-dependent interaction between cytoplasmic chaperonin and distinctive folding intermediates. Mol Cell Biol 14:2895–2904

Melki R, Vainberg IE, Chow RL, Cowan NJ (1993) Chaperonin-mediated folding of vertebrate actin-related protein and gamma-tubulin. J Cell Biol 122:1301–1310

Melki R, Batelier G, Soulie S, Williams RCJ (1997) Cytoplasmic chaperonin containing TCP-1: structural and functional characterization. Biochemistry 36:5817–5826

Meyer AS, Gillespie JR, Walther D, Millet IS, Doniach S, Frydman J (2003) Closing the folding chamber of the eukaryotic chaperonin requires the transition state of ATP hydrolysis. Cell 113:369–381

Mitchison TJ (2012) The proliferation rate paradox in antimitotic chemotherapy. Mol Biol Cell 23:1–6

Muñoz IG, Yébenes H, Zhou M, Mesa P, Serna M, Park AY, Bragado-Nilsson E, Beloso A, de Cárcer G, Malumbres M (2011) Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. Nat Struct Mol Biol 18:14–19

Munoz IG, Yebenes H, Zhou M, Mesa P, Serna M, Park AY, Bragado-Nilsson E, Beloso A, de Carcer G, Malumbres M, Robinson CV, Valpuesta JM, Montoya G (2011) Crystal structure of the open conformation of the mammalian chaperonin CCT in complex with tubulin. Nat Struct Mol Biol 18:14–19

Netzer WJ, Hartl FU (1997) Recombination of protein domains facilitated by co-translational folding in eukaryotes. Nature 388:343–349

Nicola AV, Chen W, Helenius A (1999) Co-translational folding of an alphavirus capsid protein in the cytosol of living cells. Nat Cell Biol 1:341–345

Peters JM (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. Nat Rev Mol Cell Biol 7:644–656

Pijnappel WW, Schaft D, Roguev A, Shevchenko A, Tekotte H, Wilm M, Rigaut G, Seraphin B, Aasland R, Stewart AF (2001) The S. cerevisiae SET3 complex includes two histone deacetylases, Hos2 and Hst1, and is a meiotic-specific repressor of the sporulation gene program. Genes Dev 15:2991–3004

Qian-Lin Z, Ting-Feng W, Qi-Feng C, Min-Hua Z, Ai-Guo L (2010) Inhibition of cytosolic chaperonin CCTζ-1 expression depletes proliferation of colorectal carcinoma in vitro. J Surg Oncol 102(5):419–423

Reissmann S, Parnot C, Booth CR, Chiu W, Frydman J (2007) Essential function of the built-in lid in the allosteric regulation of eukaryotic and archaeal chaperonins. Nat Struct Mol Biol 14:432–440

Reissmann S, Joachimiak LA, Chen B, Meyer AS, Nguyen A, Frydman J (2012) A gradient of ATP affinities generates an asymmetric power stroke driving the chaperonin TRIC/CCT folding cycle. Cell Rep 2:866–877

Roh SH, Kasembeli M, Galaz-Montoya JG, Trnka M, Lau WC, Burlingame A, Chiu W, Tweardy DJ (2016) Chaperonin TRiC/CCT modulates the folding and activity of leukemogenic fusion Oncoprotein AML1-ETO. J Biol Chem 291:4732–4741

Rommelaere H, de Neve M, Melki R, Vandekerckhove J, Ampe C (1999) The cytosolic class II chaperonin CCT recognizes delineated hydrophobic sequences in its target proteins. Biochemistry 38:3246–3257

Roobol A, Roobol J, Carden MJ, Smith ME, Hershey JW, Bastide A, Knight JR, Willis AE, Smales CM (2014) The chaperonin CCT interacts with and mediates the correct folding and activity of three subunits of translation initiation factor eIF3: b, i and h. Biochem J 458:213–224

Rüßmann F, Stemp MJ, Mönkemeyer L, Etchells SA, Bracher A, Hartl FU (2012) Folding of large multidomain proteins by partial encapsulation in the chaperonin TRiC/CCT. Proc Natl Acad Sci U S A 109:21208–21215

Rye HS, Burston SG, Fenton WA, Beechem JM, Xu Z, Sigler PB, Horwich AL (1997) Distinct actions of cis and trans ATP within the double ring of the chaperonin GroEL. Nature 388:792–798

Sergeeva OA, Chen B, Haase-Pettingell C, Ludtke SJ, Chiu W, King JA (2013) Human CCT4 and CCT5 chaperonin subunits expressed in Escherichia coli form biologically active homo-oligomers. J Biol Chem 288(24):17734–17744

Shi Y, Deng X, Zhan Q, Shen B, Jin X, Zhu Z, Chen H, Li H, Peng C (2013) A prospective proteomic-based study for identifying potential biomarkers for the diagnosis of cholangiocarcinoma. J Gastrointest Surg 17(9):1584–1591

Spiess C, Meyer AS, Reissmann S, Frydman J (2004) Mechanism of the eukaryotic chaperonin: protein folding in the chamber of secrets. Trends Cell Biol 14:598–604

Spiess C, Miller EJ, Mcclellan AJ, Frydman J (2006) Identification of the TRiC/CCT substrate binding sites uncovers the function of subunit diversity in eukaryotic chaperonins. Mol Cell 24:25–37

Srikakulam R, Winkelmann DA (1999) Myosin II folding is mediated by a molecular chaperonin. J Biol Chem 274:27265–27273

Sudakin V, Ganoth D, Dahan A, Heller H, Hershko J, Luca FC, Ruderman JV, Hershko A (1995) The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. Mol Biol Cell 6:185–197

Szpikowska BK, Sherman MA, Mas MT, Swiderek KM (1998) MgATP binding to the nucleotide-binding domains of the eukaryotic cytoplasmic chaperonin induces conformational changes in the putative substrate-binding domains. Protein Sci 7:1524–1530

Tomczak K, Czerwińska P, Wiznerowicz M (2015) The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) 19(1A):A68–A77

Trent JD, Nimmesgern E, Wall JS, Hartl F-U, Horwich AL (1991) A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. Nature 354:490–493

Trinidad AG, Muller PA, Cuellar J, Klejnot M, Nobis M, Valpuesta JM, Vousden KH (2013) Interaction of p53 with the CCT complex promotes protein folding and wild-type p53 activity. Mol Cell 50:805–817

Valpuesta JM, Carrascosa JL, Willison KR (2005) Structure and function of the cytosolic chaperonin CCT. In: Protein folding handbook. Wiley, Weinheim, pp 725–755

Vinh DB, Drubin DG (1994) A yeast TCP-1-like protein is required for actin function in vivo. Proc Natl Acad Sci U S A 91:9116–9120

Wells CA, Dingus J, Hildebrandt JD (2006) Role of the chaperonin CCT/TRiC complex in G protein betagamma-dimer assembly. J Biol Chem 281:20221–20232

Won KA, Schumacher RJ, Farr GW, Horwich AL, Reed SI (1998) Maturation of human cyclin E requires the

function of eukaryotic chaperonin CCT. Mol Cell Biol 18:7584–7589

Yaffe MB, Farr GW, Miklos D, Horwich AL, Sternlicht ML, Sternlicht H (1992) TCP1 complex is a molecular chaperone in tubulin biogenesis. Nature 358:245–248

Yam AY, Xia Y, Lin HT, Burlingame A, Gerstein M, Frydman J (2008) Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. Nat Struct Mol Biol 15:1255–1262

Yébenes H, Mesa P, Muñoz IG, Montoya G, Valpuesta JM (2011) Chaperonins: two rings for folding. Trends Biochem Sci 36:424–432

Yokota S, Yamamoto Y, Shimizu K, Momoi H, Kamikawa T, Yamaoka Y, Yanagi H, Yura T, Kubota H (2001) Increased expression of cytosolic chaperonin CCT in human hepatocellular and colonic carcinoma. Cell Stress Chaperones 6(4):345–350

Yuan J, Kramer A, Matthess Y, Yan R, Spankuch B, Gatje R, Knecht R, Kaufmann M, Strebhardt K (2006) Stable gene silencing of cyclin B1 in tumor cells increases susceptibility to taxol and leads to growth arrest in vivo. Oncogene 25:1753–1762

Zang Y, Jin M, Wang H, Cui Z, Kong L, Liu C, Cong Y (2016) Staggered ATP binding mechanism of eukaryotic chaperonin TRiC (CCT) revealed through high-resolution cryo-EM. Nat Struct Mol Biol 23:1083–1091

Zang Y, Wang H, Cui Z, Jin M, Liu C, Han W, Wang Y, Cong Y (2018) Development of a yeast internal-subunit eGFP labeling strategy and its application in subunit identification in eukaryotic group II chaperonin TRiC/CCT. Sci Rep 8:2374

Zebol JR, Hewitt NM, Moretti PA, Lynn HE, Lake JA, Li P, Vadas MA, Wattenberg BW, Pitson SM (2009) The CCT/TRiC chaperonin is required for maturation of sphingosine kinase 1. Int J Biochem Cell Biol 41:822–827

Zhang J, Wu X, Zan J, Wu Y, Ye C, Ruan X, Zhou J (2013) Cellular chaperonin CCTgamma contributes to rabies virus replication during infection. J Virol 87:7608–7621

Zhang Y, Wang Y, Wei Y, Wu J, Zhang P, Shen S, Saiyin H, Wumaier R, Yang X, Wang C (2016) Molecular chaperone CCT3 supports proper mitotic progression and cell proliferation in hepatocellular carcinoma cells. Cancer Lett 372:101–109

Regulation of Hsf1 and the Heat Shock Response

3

David Pincus

Abstract

The heat shock response (HSR) is characterized by the induction of molecular chaperones following a sudden increase in temperature. In eukaryotes, the HSR comprises the set of genes controlled by the transcription factor Hsf1. The HSR is induced by defects in co-translational protein folding, ribosome biogenesis, organellar targeting of nascent proteins, and protein degradation by the ubiquitin proteasome system. Upon heat shock, these processes may be endogenous sources of polypeptide ligands that activate the HSR. Mechanistically, these ligands are thought to titrate the chaperone Hsp70 away from Hsf1, releasing Hsf1 to induce the full arsenal of cellular chaperones to restore protein homeostasis. In metazoans, this cell-autonomous feedback loop is modulated by the microenvironment and neuronal cues to enable tissue-level and organism-wide coordination.

Keywords

Hsf1 · Heat shock · Heat shock protein · Chaperone · Proteostasis · Hsp70

3.1 Introduction: The HSR in Health and Disease

The heat shock response (HSR) is conserved in all kingdoms of life and is characterized by the induction of molecular chaperones following a sudden increase in temperature. Initially observed as heat-induced chromosomal puffs in *Drosophila*, the HSR has long served as a model system for studying the molecular mechanisms of inducible transcription (Anckar and Sistonen 2011). In recent years, as protein homeostasis (proteostasis) has become increasingly implicated in cancer, neurodegenerative disease and aging, studies of the HSR have focused on its role as the regulatory nexus for the proteostasis network (Labbadia and Morimoto 2014).

In eukaryotes, the HSR is regulated by a conserved family of heat shock transcription factors (HSFs). HSFs are winged helix-loop-helix DNA binding proteins that trimerize and recognize a conserved motif found in the promoters of chaperone genes (Hentze et al. 2016; Littlefield and Nelson 1999; Neudegger et al. 2016; Sorger and Nelson 1989). Yeast and invertebrates have a single HSF – Hsf1 – while mammalian genomes

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_3

encode Hsf1 along with Hsf2 and additional tissue-specific paralogs implicated in development (Anckar and Sistonen 2011). In the absence of Hsf1, mouse embryonic fibroblasts (MEFs) fail to induce chaperone genes following heat shock, indicating that Hsf1 is primarily responsible for regulating the canonical HSR in mammalian cells (Mahat et al. 2016; McMillan et al. 1998; Solís et al. 2016). However, Hsf2 can hetero-oligomerize with Hsf1, and MEFs lacking Hsf2 show increased basal expression and altered induction of HSR targets, suggesting that Hsf2 may modulate Hsf1 (Jaeger et al. 2016; Östling et al. 2007; Solís et al. 2016). Consistent with an antagonistic interaction, Hsf1 has been shown to promote survival and malignancy in cancer models, while Hsf2 suppresses tumor progression (Björk et al. 2016).

Cancer cells rely on the HSR to support rapid growth and to counteract the deleterious consequences of high mutational loads (Dai 2018; Dai and Sampson 2016). In many human tumor samples and cancer cell lines, Hsf1 is constitutively activated and required for proliferation (Dai et al. 2007; Santagata et al. 2011). High levels of Hsf1 in tumor samples – both in the tumor cells as well as in the supporting stromal cells – is correlated with poor prognosis in several cohorts of cancer patients (Santagata et al. 2011; Scherz-Shouval et al. 2014). Moreover, Hsf1 knock out mice are resistant to tumor growth (Dai and Sampson 2016; Dai et al. 2007; 2012; Min et al. 2007). Based on these observations, inhibiting Hsf1 has been proposed as an anticancer strategy (Whitesell and Lindquist 2009).

Conversely, activating Hsf1 has been proposed as a therapeutic strategy to combat neurodegenerative diseases (Neef et al. 2011). Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, frontotemporal dementia, and Huntington's disease all share protein aggregation as a hallmark (Soto 2003). Chaperone levels are known to decline in the brain with age, and Hsf1 undergoes abnormal degradation in cell line and mouse models of Huntington's disease (Brehme et al. 2014; Gomez-Pastor et al. 2017; Yang et al. 2014). Moreover, activation of Hsf1 by a natural product ameliorates phenotypes associated with polyglutamine expansion diseases in *Drosophila* (Nelson et al. 2014). Thus, enhancing Hsf1 activity could induce the HSR to prevent or reverse protein aggregation and slow neurodegeneration.

The central and opposing roles for Hsf1 in cancer and neurodegenerative diseases suggest that therapeutic intervention into the HSR may involve an inherent trade-off, so quantitative and dynamic control would be highly valuable. Understanding the mechanisms that regulate Hsf1 in healthy cells may reveal the processes that break down and are hijacked in disease.

3.2 Defining the Transcriptional Response to Heat Shock

Counterintuitively, the HSR comprises only a fraction of genes that change expression upon heat shock. In yeast, hundreds of genes are induced following an increase in temperature, and hundreds more are repressed (Gasch et al. 2000; Solís et al. 2016). However, Hsf1 controls expression of a dedicated proteostasis regulon containing fewer than 50 of the induced genes and has no role in transcriptional repression (Pincus et al. 2018; Solís et al. 2016). In addition to robust transcriptional activation, Hsf1 drives intergenic interactions among its target gene loci during heat shock and remodels the three-dimensional architecture of the yeast genome (Chowdhary et al. 2017; 2019). The remainder of the differentially expressed genes comprise the environmental stress response (ESR), a generic program activated by a variety of environmental perturbations. The induced ESR is controlled by the general stress response transcription factors Msn2/4, while the repressed ESR is enriched for highly expressed genes encoding factors involved in central metabolism and ribosome biogenesis (Gasch and Werner-Washburne 2002). Heat shock-dependent repression of highly expressed genes occurs in *Drosophila* and mammalian cells too and is also Hsf1-independent (Duarte et al. 2016; Mahat et al. 2016). Thus, an additional conserved aspect of the transcriptional response to elevated temperature is repression of highly expressed genes (Anckar and Sistonen 2011). As in yeast, Hsf1 only controls a fraction of the

genes induced by heat shock in mammalian cells (Mahat et al. 2016; Solís et al. 2016). Nevertheless, the HSR has come to be defined as the set of genes induced by Hsf1. Using this definition, the HSR is highly conserved across eukaryotes and limited to genes encoding chaperones and other proteostasis factors.

However, the HSR is not so clearly defined in the context of multicellular development, metabolism and cancer. Hsf1 is known to drive transcriptional programs distinct from the canonical HSR during development and inflammation, in animal models of obesity, and in highly malignant cancer cells (Ali et al. 2018; Li et al. 2016; Ma et al. 2015; Mendillo et al. 2012). In addition to proteostasis factors, Hsf1 controls cell cycle and metabolic genes that promote oncogenesis in multiple tumor types (Mendillo et al. 2012). Hsf1 is also active in cancer associated fibroblasts in the stroma surrounding tumors and drives another distinct transcriptional program in these cells that promotes tumor growth via paracrine signaling (Scherz-Shouval et al. 2014). It is not yet clear how Hsf1 is directed to alternative sites in the genome to regulate these noncanonical transcriptional programs.

3.3 What Activates the HSR?

Despite its name, the HSR is not exclusively sensitive to temperature. Oxidative stress, glucose depletion and overexpression of a constitutively misfolded protein also activate Hsf1 and induce chaperones, as do several classes of small molecules including Hsp90 inhibitors, proteasome inhibitors, amino acid analogs, and ribosome biogenesis inhibitors (Alford and Brandman 2018; Geiler-Samerotte et al. 2011; Hahn and Thiele 2004; Kim et al. 1999a, b; Trotter et al. 2002; Tye et al. 2019; Yamamoto et al. 2007). At the molecular level, these inputs converge on Hsf1.

It is often presumed that heat shock – and by extension the set of other environmental and chemical perturbations that activate Hsf1 – causes a fraction of the mature proteome to denature and aggregate, and these aggregates serve as the molecular signals that induce the HSR (Lindquist 1986). At the biochemical level, differential centrifugation experiments have shown that proteins sediment in high molecular weight fractions during heat shock in a manner that is partially reversible by molecular chaperones, suggesting heat shock-dependent protein aggregation (Mogk et al. 1999). But it is now appreciated that enzymes in these aggregates can retain activity, suggesting that these assemblies do not contain denatured or misfolded proteins (Riback et al. 2015; Wallace et al. 2015). At the cell biological level, observations in yeast and mammalian cells revealed that in response to heat shock, chaperones form subcellular foci that colocalize with aggregation-prone reporter proteins, and chaperones form similar puncta during heat shock in the absence of reporters (Cherkasov et al. 2013; Kaganovich et al. 2008; Solís et al. 2016). These results were interpreted to suggest that endogenous metastable proteins denature and are also in these puncta. However, no such endogenous proteins have been identified.

Genome-wide deletion and RNAi screens were conducted in yeast and human cells to identify genes involved in regulating a reporter gene under the control of Hsf1. The yeast screen identified genes that when deleted altered reporter levels at 25 and 37 °C, while the human screen revealed factors that modulated induction of the reporter following heat shock and recovery, mostly identifying genes required for activation (Brandman et al. 2012; Raychaudhuri et al. 2014). Both screens implicated the proteasome in negatively regulating Hsf1 activity, while the top hits in the yeast screen also included chaperones, organelle targeting machinery, and the ribosome quality control complex (RQC) as negative regulators of Hsf1. Chemical genetic experiments revealed that acute depletion of ribosome biogenesis factors also results in potent Hsf1 activation due to the accumulation of orphan ribosomal proteins (Albert et al. 2019; Tye et al. 2019). Synthetic mutant proteins that clog ER and mitochondrial import pathways likewise activate Hsf1 (Boos et al. 2019; Shmidt et al. 2019). A unifying theme among these mutants is that proteins are likely to accumulate in the cell that are not present in wild type cells: ubiquitin proteasome sys-

tem (UPS) intermediates, unbound chaperone clients, mistargeted organellar proteins, partially-translated 60S ribosome-bound nascent chains, and unincorporated ribosomal proteins (Fig. 3.1).

Do these genetic results support the protein aggregate model? Rather than implicating denaturation of the mature proteome, the genetics suggest that Hsf1 is sensitive to dynamic aspects proteostasis: nascent chain folding, protein complex formation, ribosome biogenesis, post-translational organelle targeting, and degradation. Consistent with this notion, pretreatment with cycloheximide to stop translation prior to heat shock abolishes HSR induction (Baler et al. 1992). Moreover, a small molecule screen in human cells for modulators of a reporter gene of Hsf1 activity revealed that broad classes of translation inhibitors prevent HSR activation (Santagata et al. 2013). Taken together, these biochemical, cell biological, genetic, and pharmacological experiments suggest that the HSR does indeed monitor proteostasis. However, it is unlikely to be the case that mature proteins denature *en masse* upon heat shock, and the resulting aggregates activate Hsf1. Rather, Hsf1 appears to respond to stalled ribosomes recognized by RQC, orphan ribosomal proteins that result from aborted ribosome biogenesis, clogged ER and mitochondrial import machinery, and an overtaxed UPS. Thus, the HSR seems tuned to surveil the early and late events in the life of proteins rather than the mature proteome.

3.4 Hsp70 and Hsf1 Constitute a Negative Feedback Loop That Controls the HSR

How are inefficiencies in protein complex formation, ribosome biogenesis, organelle targeting, and degradation communicated to Hsf1 to induce the HSR? Based on observations in *Drosophila*, the HSR is canonically thought to be an autoregulatory feedback loop for heat shock protein (HSP)

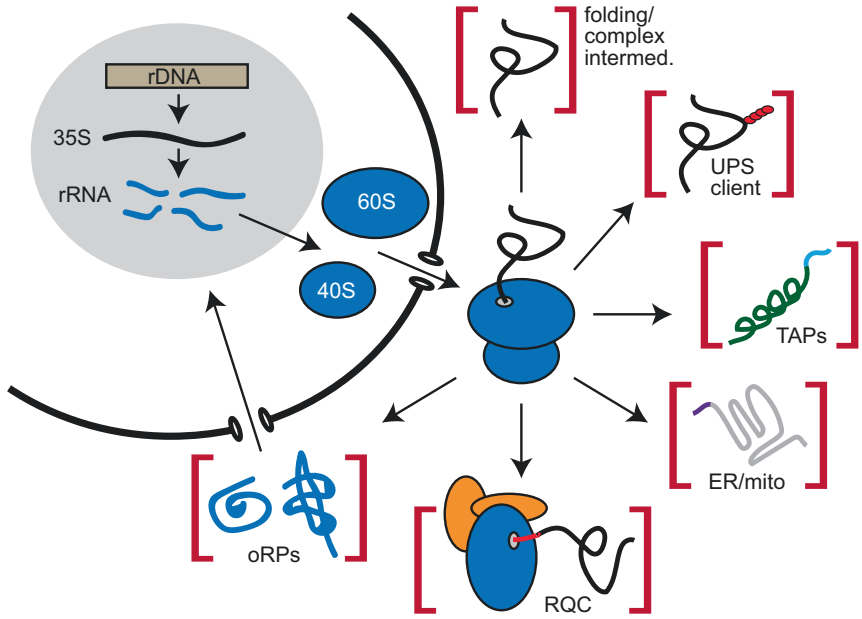

Fig. 3.1 Sources of ligands for the HSR. The canonical HSR is induced by defects in a variety of cell biological processes. These include nascent protein folding and complex formation, ribosome biogenesis (leading to accumulation of orphan ribosomal proteins, oRPs), ribosome quality control (RQC), ER and mitochondrial targeting, tail anchored protein (TAP) insertion, degradation by the ubiquitin/proteasome system (UPS). A common theme among these processes is that their disruption results in the accumulation of proteins in the cytosol that are not supposed to be there

expression (Didomenico et al. 1982; Solomon et al. 1991). In this model, often referred to as the chaperone titration model, excess HSPs bind to and repress a transcription factor. When conditions change and the cell needs more chaperones – i.e., when HSPs become limiting and are titrated away – the transcription factor is free to induce more HSPs until they are in excess again.

The mechanistic precedent for the chaperone titration model was established in *E. coli*. In this prokaryotic system, the homolog of the Hsp70 chaperone (DnaK) represses the heat shock transcription factor σ32 by binding and accelerating its degradation; when the levels of DnaK become limiting, σ32 accumulates and induces transcription of DnaK along with the rest of the HSR (Bukau and Walker 1990; Straus et al. 1990). In yeast, molecular genetic experiments also suggested that Hsp70 autoregulated its own expression: mutation of the two highly expressed Hsp70 paralogs (*ssa1 ssa2*) results in induction of a third Hsp70 paralog (*SSA3*), and this induction requires the heat shock element (HSE) – the conserved binding site for Hsf1 – in the *SSA3* promoter (Boorstein and Craig 1990). In human cells, the genes encoding Hsp70 also contain HSEs in their promoters, and the Hsp70 protein directly binds to Hsf1 and impairs its ability associate with HSE-containing DNA (Shi et al. 1998). Taken together, these data support a model in which the HSR is an autoregulatory loop controlled by Hsp70 in both prokaryotic and eukaryotic cells.

Affinity purification experiments coupled to mass spectrometry using Hsf1 as bait revealed a specific and dynamic interaction between Hsp70 and Hsf1 in yeast cells (Zheng et al. 2016). Hsf1 has also been shown to directly crosslink to the Hsp70 substrate binding domain under nonstress conditions and dissociate during heat shock and other genetic and chemical perturbations to proteostasis (Masser et al. 2019). Under basal conditions, Hsf1 co-precipitates with Hsp70; the interaction is lost following acute heat shock; over sustained heat shock, the interaction is restored. The dynamics of the Hsp70:Hsf1 interaction are the mirror image of Hsf1-dependent transcription, which is transiently increased during heat shock (Zheng et al. 2016). Mutational analysis and

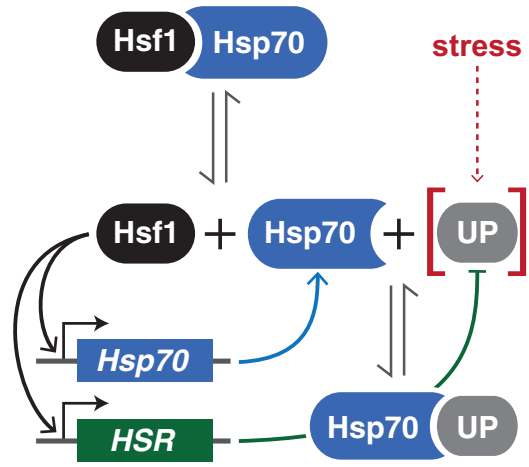

Fig. 3.2 Hsp70 and Hsf1 form a negative feedback loop that controls the HSR. Hsp70 binds and represses Hsf1 under basal conditions. Heat shock and other proteotoxic stress conditions generate unstable polypeptides (UPs) – the ligands of the HSR depicted in Fig. 3.1. UPs titrate Hsp70 away from Hsf1, leaving Hsf1 free to induce transcription of Hsp70 and the rest of the HSR target genes. Once the UPs have been cleared, Hsp70 is again in excess and can bind and deactivate Hsf1

biochemical binding assays revealed a specific Hsp70 binding site on Hsf1 known as conserved element 2 (CE2) (Krakowiak et al. 2018). CE2 is required for Hsf1 repression under basal conditions and deactivation of Hsf1 following heat shock. In addition, a second Hsp70 binding site has been identified in the N-terminal region of Hsf1 (Peffer et al. 2019). Transcriptional induction of Hsp70 is also required for appropriate regulation of the HSR, as HSE disruption in the promoters of the Hsp70 genes impairs Hsf1 deactivation following heat shock (Krakowiak et al. 2018). Thus, Hsp70 and Hsf1 form a negative feedback loop in which Hsf1 induces Hsp70 expression, and Hsp70 represses Hsf1 activity (Fig. 3.2).

3.5 Hsp90 Negatively Regulates Hsf1 Orthogonally to Hsp70

Pharmacological and genetic experiments also demonstrated that impaired Hsp90 function activates Hsf1 (Brandman et al. 2012; Kim et al. 1999b). Hsp90 was shown to bind to Hsf1 in

mammalian cell lysate and has also been proposed to regulate Hsf1 via a titration model (Zou et al. 1998). In yeast, deletion of the highly expressed Hsp90 paralog induces Hsf1 activation, but no protein-protein interaction has been reported between Hsp90 and Hsf1. Recently, use of a novel reporter of Hsp90 availability revealed that Hsp90 represses Hsf1 in a manner that is independent of Hsp70 (Alford and Brandman 2018). The mechanism by which this orthogonal Hsp90 axis regulates Hsf1 remains unknown.

3.6 Phosphorylation Is Dispensable for Hsf1 Activity During Heat Shock

In addition to regulation by HSPs, Hsf1 is also post-translationally modified in response to heat shock. Hsf1 has been shown to be ubiquitylated by FBXW7, resulting in degradation by the proteasome, as well as SUMOylated and acetylated in human cells (Hong et al. 2001; Kourtis et al. 2015; Westerheide et al. 2009). Hsf1 has also been shown to be phosphorylated in diverse eukaryotes (Anckar and Sistonen 2011; Sorger and Pelham 1988). In yeast, there is evidence that Hsf1 can be phosphorylated on 73 distinct sites, and 15 phosphorylation sites were identified in the regulatory domain of human Hsf1 (Budzyński et al. 2015; Zheng et al. 2016). In both cases, however, mutational analysis revealed that simultaneous mutation of all sites to alanine – resulting in mutants that cannot be phosphorylated – had minimal effects on the ability of Hsf1 to activate the HSR in response to heat shock. In other words, phosphorylation is dispensable for activation of Hsf1. However, a mutant that mimics constitutive hyperphosphorylation (via substitution of negatively charged amino acids) is highly active under basal conditions in yeast (Zheng et al. 2016). Thus, while phosphorylation is not necessary for Hsf1 activation, negative charge is sufficient. Neither the phosphorylation-deficient mutant nor the phospho-mimetic mutant altered the interaction between Hsf1 and Hsp70 in yeast (Zheng et al. 2016). Thus, like Hsp90 inhibition, phosphorylation represents a regulatory axis orthogonal to the Hsp70 feedback loop.

While dispensable for activation in response to heat shock, single cell measurements revealed that Hsf1 phosphorylation promotes cell-to-cell variation in the HSR in yeast. Variation in the expression of Hsp90 driven by Hsf1 phosphorylation enables cells to acquire resistance to an antifungal drug (Zheng et al. 2018). Hsp90 is known to promote phenotypic diversity and has been proposed to play important roles in molecular evolution (Lindquist 2009). By generating variation in Hsp90 levels across a population, Hsf1 phosphorylation may be advantageous for cells in fluctuating environmental conditions.

Despite the minimal role of Hsf1 phosphorylation sites following heat shock, multiple kinases have been implicated in Hsf1 phosphorylation in yeast and mammalian cells. In human cells, MEK has been shown to promote Hsf1 activation, while AMPK inhibits Hsf1 (Dai et al. 2015; Tang et al. 2015). In addition, ERK, GSK3β and CK2α′ phosphorylate Hsf1 to target it to the UPS for degradation (Li et al. 2017). In yeast, the AMPK homolog Snf1 has also been shown to phosphorylate Hsf1 and modulate the HSR (Hahn and Thiele 2004). The discrepancy between these kinase-mediated regulatory events and the ability of Hsf1 to be activated in the absence of phosphorylation is unresolved. It is clear that Hsf1 becomes phosphorylated during heat shock, but it is not the simple case that phosphorylation is required to activate Hsf1 following heat shock. Phosphorylation may be an important mode of Hsf1 regulation in response to signals other than heat shock.

3.7 Coordination of the HSR Across Tissues

In multicellular animals, homeostasis is a property of the organism rather than the individual cell. As such, cell-autonomous Hsf1 regulatory mechanisms are augmented by intercellular coordination in metazoans (Fig. 3.3). For example, insulin/IGF-1 signaling is known to modulate Hsf1 activity in *C. elegans* to regulate lifespan via Hsf1-dependent control of cytoskeletal integrity (Baird et al. 2014; Hsu et al. 2003). Moreover, at the onset of reproductive maturity in *C. ele-*

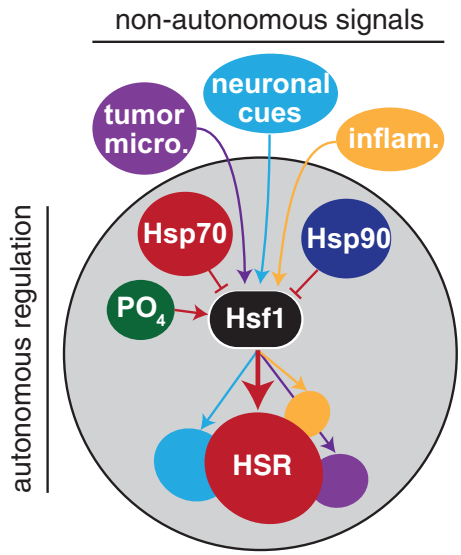

Fig. 3.3 Non-autonomous regulation of Hsf1 results in non-canonical HSR induction. In addition to the cell-autonomous regulatory mechanisms that control Hsf1 activity to control the canonical HSR target genes (red arrows), metazoans can regulate Hsf1 and the HSR via non-autonomous signals. The tumor microenvironment, inflammatory cytokines, dietary hormones, and neuronal signaling has been shown to involve Hsf1. Once activated by these extracellular signals, Hsf1 can initiate distinct transcriptional programs that only partially overlap with the canonical HSR

gans, signals from germ stem cells result in organism-wide inactivation of the HSR by epigenetic silencing of target gene promoters (Labbadia and Morimoto 2015). In mammals, as described above, the tumor microenvironment and organism-wide metabolic signaling subjugate Hsf1 and the transcriptional program it controls (Ma et al. 2015; Scherz-Shouval et al. 2014). These forms of non-autonomous regulation are mediated by hormones, cytokines and growth factors. In most cases, it is not yet understood how the signaling cascades activated by these ligands impinge on Hsf1, nor how Hsf1 is then deployed to regulate distinct target genes.

In addition to paracrine and endocrine signaling, neuronal signaling in *C. elegans* is required for organism-wide induction of the HSR in response to temperature (Prahlad et al. 2008). Indeed, separate Hsf1-dependent neural signals have been shown to be responsible for mediating heat shock signaling and longevity (Douglas et al. 2015). Moreover, local ectopic expression of a misfolded protein in muscle cells was shown to induce the HSR across multiple tissue types, and local over-expression of Hsp90 in neurons or intestinal cells suppressed HSR induction arising from proteostasis defects in muscle cells (van Oosten-Hawle et al. 2013). These results indicate that both forward stress signaling and chaperone-mediated feedback control operate across tissues.

3.8 Conclusion

The HSR is both highly conserved and remarkably plastic. In eukaryotes from yeast to humans, the same core set of chaperone-encoding genes is induced by heat shock, and these genes harbor the same *cis*-acting motif in their promoters that is recognized by the same sequence-specific DNA binding protein, Hsf1. Approaches from genetics, chemical biology, biochemistry and cell biology reveal a coherent picture of the molecular consequences of heat shock and the regulatory mechanisms that govern Hsf1 activity. Rather than global protein denaturation, heat shock is likely to impair key biogenesis processes like protein complex formation and ribosome production, resulting in the accumulation of orphan subunits and other unstable polypeptides that are sequestered and/or degraded via chaperones. Heat shock, or disruption of these biogenesis and degradation pathways by genetic or pharmacological means, activates Hsf1 principally by titrating the chaperone Hsp70 away from its repressive interaction with Hsf1. Phosphorylation and Hsp90 also regulate Hsf1 activity, but the mechanisms remain unresolved. While these core cell-autonomous regulatory mechanisms are conserved over evolution, metazoans have expanded both the input signals that activate Hsf1 as well as the target genes that Hsf1 controls. While this plasticity in the HSR enables the proteostasis network to incorporate signals from other cells and allows Hsf1 to activate distinct transcriptional programs, this plasticity may also permit tumor cells to hijack the HSR. However, the deep conservation of the cell-autonomous regulatory mechanisms may allow for the development of targeted therapeutics that will allow us to take back control.

Acknowledgements Thanks to A. Ali and G. Bushkin for critical reading of the manuscript. This work was supported by an Early Independence Award from the NIH (DP5 OD017941).

References

Albert B, Kos-Braun IC, Henras AK, Dez C, Rueda MP, Zhang X, Gadal O, Kos M, Shore D (2019) A ribosome assembly stress response regulates transcription to maintain proteome homeostasis. Elife 8:e45002

Alford BD, Brandman O (2018) Quantification of Hsp90 availability reveals differential coupling to the heat shock response. J Cell Biol 217:3809–3816

Ali A, Biswas A, Pal M (2018) HSF1 mediated TNF-α production during proteotoxic stress response pioneers proinflammatory signal in human cells. FASEB J 33:2621–2635

Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80:1089–1115

Baird NA, Douglas PM, Simic MS, Grant AR, Moresco JJ, Wolff SC, Yates JR, Manning G, Dillin A (2014) HSF-1–mediated cytoskeletal integrity determines thermotolerance and life span. Science 346:360–363

Baler R, Welch W, Voellmy R (1992) Heat shock gene regulation by nascent polypeptides and denatured proteins: hsp70 as a potential autoregulatory factor. J Cell Biol 117:1151–1159

Björk J, Åkerfelt M, Joutsen J, Puustinen M, Cheng F, Sistonen L, Nees M (2016) Heat-shock factor 2 is a suppressor of prostate cancer invasion. Oncogene 35:1770

Boorstein W, Craig E (1990) Transcriptional regulation of SSA3, an HSP70 gene from Saccharomyces cerevisiae. Mol Cell Biol 10:3262–3267

Boos F, Krämer L, Groh C, Jung F, Haberkant P, Stein F, Wollweber F, Gackstatter A, Zöller E, van der Laan M et al (2019) Mitochondrial protein-induced stress triggers a global adaptive transcriptional programme. Nat Cell Biol 21(4):442–451

Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li G-W, Zhou S, King D, Shen PS, Weibezahn J et al (2012) A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. Cell 151:1042–1054

Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, Orton K, Villella A, Garza D, Vidal M et al (2014) A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep 9:1135–1150

Budzyński MA, Puustinen MC, Joutsen J, Sistonen L (2015) Uncoupling stress-inducible phosphorylation of heat shock factor 1 from its activation. Mol Cell Biol 35:2530–2540

Bukau B, Walker G (1990) Mutations altering heat shock specific subunit of RNA polymerase suppress major cellular defects of E. coli mutants lacking the DnaK chaperone. EMBO J 9:4027–4036

Cherkasov V, Hofmann S, Druffel-Augustin S, Mogk A, Tyedmers J, Stoecklin G, Bukau B (2013) Coordination of translational control and protein homeostasis during severe heat stress. Curr Biol 23:2452–2462

Chowdhary S, Kainth AS, Gross DS (2017) Heat shock protein genes undergo dynamic alteration in their three-dimensional structure and genome organization in response to thermal stress. Mol Cell Biol 37:e00292-17

Chowdhary S, Kainth AS, Pincus D, Gross DS (2019) Heat shock factor 1 drives intergenic association of its target gene loci upon heat shock. Cell Rep 26:18–28.e5

Dai C (2018) The heat-shock, or HSF1-mediated proteotoxic stress, response in cancer: from proteomic stability to oncogenesis. Philos Trans R Soc Lond B Biol Sci 373:20160525

Dai C, Sampson S (2016) HSF1: guardian of proteostasis in cancer. Trends Cell Biol 26:17–28

Dai C, Whitesell L, Rogers AB, Lindquist S (2007) Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. Cell 130:1005–1018

Dai C, Santagata S, Tang Z, Shi J, Cao J, Kwon H, Bronson RT, Whitesell L, Lindquist S (2012) Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. J Clin Invest 122:3742–3754

Dai S, Tang Z, Cao J, Zhou W, Li H, Sampson S, Dai C (2015) Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. EMBO J 34:275–293

Didomenico BJ, Bugaisky GE, Lindquist S (1982) The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Cell 31:593–603

Douglas PM, Baird NA, Simic MS, Uhlein S, McCormick MA, Wolff SC, Kennedy BK, Dillin A (2015) Heterotypic signals from neural HSF-1 separate thermotolerance from longevity. Cell Rep 12:1196–1204

Duarte FM, Fuda NJ, Mahat DB, Core LJ, Guertin MJ, Lis JT (2016) Transcription factors GAF and HSF act at distinct regulatory steps to modulate stress-induced gene activation. Genes Dev 30:1731–1746

Gasch AP, Werner-Washburne M (2002) The genomics of yeast responses to environmental stress and starvation. Funct Integr Genomics 2:181–192

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257

Geiler-Samerotte KA, Dion MF, Budnik BA, Wang SM, Hartl DL, Drummond AD (2011) Misfolded proteins impose a dosage-dependent fitness cost and trigger a cytosolic unfolded protein response in yeast. Proc National Acad Sci 108:680–685

Gomez-Pastor R, Burchfiel ET, Neef DW, Jaeger AM, Cabiscol E, McKinstry SU, Doss A, Aballay A, Lo

DC, Akimov SS et al (2017) Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. Nat Commun 8:14405

Hahn J-S, Thiele DJ (2004) Activation of the Saccharomyces cerevisiae heat shock transcription factor under glucose starvation conditions by Snf1 protein kinase. J Biol Chem 279:5169–5176

Hentze N, Breton L, Wiesner J, Kempf G, Mayer MP (2016) Molecular mechanism of thermosensory function of human heat shock transcription factor Hsf1. Elife 5:e11576

Hong Y, Rogers R, Matunis M, Mayhew C, Goodson M, Park-Sarge O, Sarge K, Goodson M (2001) Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. J Biol Chem 276:40263–40267

Hsu A-L, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300:1142–1145

Jaeger AM, Charles PW, Sistonen L, Thiele DJ (2016) Structures of HSF2 reveal mechanisms for differential regulation of human heat-shock factors. Nat Struct Mol Biol 23:147–154

Kaganovich D, Kopito R, Frydman J (2008) Misfolded proteins partition between two distinct quality control compartments. Nature 454:1088

Kim D, Kim S-H, Li GC (1999a) Proteasome inhibitors MG132 and lactacystin hyperphosphorylate HSF1 and induce hsp70 and hsp27 expression. Biochem Biophys Res Commun 254:264–268

Kim H, Kang H, Kim H (1999b) Geldanamycin induces heat shock protein expression through activation of HSF1 in K562 erythroleukemic cells. IUBMB Life 48:429–433

Kourtis N, Moubarak RS, Aranda-Orgilles B, Lui K, Aydin IT, Trimarchi T, Darvishian F, Salvaggio C, Zhong J, Bhatt K et al (2015) FBXW7 modulates cellular stress response and metastatic potential through HSF1 post-translational modification. Nat Cell Biol 17:322–332

Krakowiak J, Zheng X, Patel N, Feder ZA, Anandhakumar J, Valerius K, Gross DS, Khalil AS, Pincus D (2018) Hsf1 and Hsp70 constitute a two-component feedback loop that regulates the yeast heat shock response. Elife 7:e31668

Labbadia J, Morimoto RI (2014) The biology of proteostasis in aging and disease. Annu Rev Biochem 84:1–30

Labbadia J, Morimoto RI (2015) Repression of the heat shock response is a programmed event at the onset of reproduction. Mol Cell 59:639–650

Li J, Chauve L, Phelps G, Brielmann RM, Morimoto RI (2016) E2F coregulates an essential HSF developmental program that is distinct from the heat-shock response. Genes Dev 30:2062–2075

Li J, Labbadia J, Morimoto RI (2017) Rethinking HSF1 in stress, development, and organismal health. Trends Cell Biol 27:895–905

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191

Lindquist S (2009) Protein folding sculpting evolutionary change. Cold Spring Harb Symp Quant Biol 74:103–108

Littlefield O, Nelson H (1999) A new use for the "wing" of the "winged" helix-turn-helix motif in the HSF–DNA cocrystal. Nat Struct Mol Biol 6:464–470

Ma X, Xu L, Alberobello A, Gavrilova O, Bagattin A, Skarulis M, Liu J, Finkel T, Mueller E (2015) Celastrol protects against obesity and metabolic dysfunction through activation of a HSF1-PGC1α transcriptional Axis. Cell Metab 22:695–708

Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT (2016) Mammalian heat shock response and mechanisms underlying its genome-wide transcriptional regulation. Mol Cell 62:63–78

Masser AE, Kang W, Roy J, Mohanakrishnan Kaimal J, Quintana-Cordero J, Friedländer MR, Andréasson C (2019) Cytoplasmic protein misfolding titrates Hsp70 to activate nuclear Hsf1. ELife 8. pii: e47791. PMID: 31552827

McMillan RD, Xiao X, Shao L, Graves K, Benjamin IJ (1998) Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. J Biol Chem 273:7523–7528

Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, Fraenkel E, Ince TA, Whitesell L, Lindquist S (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. Cell 150:549–562

Min JN, Huang L, Zimonjic DB, Moskophidis D, Mivechi NF (2007) Selective suppression of lymphomas by functional loss of Hsf1 in a p53-deficient mouse model for spontaneous tumors. Oncogene 26:5086–5097

Mogk A, Tomoyasu T, Goloubinoff P, Rüdiger S, Röder D, Langen H, Bukau B (1999) Identification of thermolabile Escherichia coli proteins: prevention and reversion of aggregation by DnaK and ClpB. EMBO J 18:6934–6949

Neef DW, Jaeger AM, Thiele DJ (2011) Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. Nat Rev Drug Discov 10:930

Nelson VK, Ali A, Dutta N, Ghosh S, Jana M, Ganguli A, Komarov A, Paul S, Dwivedi V, Chatterjee S et al (2014) Azadiradione ameliorates polyglutamine expansion disease in Drosophila by potentiating DNA binding activity of heat shock factor 1. Oncotarget 5

Neudegger T, Verghese J, Hayer-Hartl M, Hartl UF, Bracher A (2016) Structure of human heat-shock transcription factor 1 in complex with DNA. Nat Struct Mol Biol 23:140–146

Östling P, Björk JK, Roos-Mattjus P, Mezger V, Sistonen L (2007) Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. J Biol Chem 282:7077–7086

Peffer S, Goncalves D, Morano KA (2019) Regulation of the Hsf1-dependent transcriptome via conserved bipartite contacts with Hsp70 promotes survival in yeast. J Biol Chem 294(32):12191–12202. PMID: 31239354

Pincus D, Anandhakumar J, Thiru P, Guertin MJ, Erkine AM, Gross DS (2018) Genetic and epigenetic determinants establish a continuum of Hsf1 occupancy and activity across the yeast genome. Mol Biol Cell 29(26):3168–3182

Prahlad V, Cornelius T, Morimoto RI (2008) Regulation of the cellular heat shock response in *Caenorhabditis elegans* by thermosensory neurons. Science 320:811–814

Raychaudhuri S, Loew C, Körner R, Pinkert S, Theis M, Hayer-Hartl M, Buchholz F, Hartl UF (2014) Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. Cell 156:975–985

Riback J, Laskowski P, Scott JL, Wallace E, Rojek AE, Schwartz MH, Sosnick TR, Drummond AD (2015) Heat shock triggers assembly of tRNA synthetases into an active supercomplex. Biophys J 108:221a

Santagata S, Hu R, Lin NU, Mendillo ML, Collins LC, Hankinson SE, Schnitt SJ, Whitesell L, Tamimi RM, Lindquist S et al (2011) High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proc National Acad Sci 108:18378–18383

Santagata S, Mendillo ML, Tang Y, Subramanian A, Perley CC, Roche SP, Wong B, Narayan R, Kwon H, Koeva M et al (2013) Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. Science 341:1238303

Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, Beck AH, Dias-Santagata D, Koeva M, Stemmer SM, Whitesell L et al (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. Cell 158:564–578

Shi Y, Mosser DD, Morimoto RI (1998) Molecularchaperones as HSF1-specific transcriptional repressors. Genes Dev 12:654–666

Shmidt R, Schessner JP, Borner G, Schuck S (2019) The proteasome biogenesis regulator Rpn4 cooperates with the unfolded protein response to promote ER stress resistance. Elife 8:pii: e43244

Solís EJ, Pandey JP, Zheng X, Jin DX, Gupta PB, Airoldi EM, Pincus D, Denic V (2016) Defining the essential function of yeast Hsf1 reveals a compact transcriptional program for maintaining eukaryotic proteostasis. Mol Cell 63:60–71

Solomon J, Rossi J, Golic K, McGarry T, Lindquist S (1991) Changes in hsp70 alter thermotolerance and heat-shock regulation in Drosophila. New Biol 3:1106–1120

Sorger PK, Nelson H (1989) Trimerization of a yeast transcriptional activator via a coiled-coil motif. Cell 59:807–813

Sorger PK, Pelham H (1988) Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell 54:855–864

Soto C (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 4(1):49–60

Straus D, Walter W, Gross C (1990) DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of sigma 32. Genes Dev 4:2202–2209

Tang Z, Dai S, He Y, Doty RA, Shultz LD, Sampson S, Dai C (2015) MEK guards proteome stability and inhibits tumor-suppressive amyloidogenesis via HSF1. Cell 160:729–744

Trotter EW, Kao C, Berenfeld L, Botstein D, Petsko GA, Gray JV (2002) Misfolded proteins are competent to mediate a subset of the responses to heat shock in Saccharomyces cerevisiae. J Biol Chem 277:44817–44825

Tye BW, Commins N, Ryazanova LV, Wühr M, Springer M, Pincus D, Churchman SL (2019) Proteotoxicity from aberrant ribosome biogenesis compromises cell fitness. Elife 8:43002

van Oosten-Hawle P, Porter RS, Morimoto RI (2013) Regulation of organismal proteostasis by transcellular chaperone signaling. Cell 153:1366–1378

Wallace E, Kear-Scott JL, Pilipenko EV, Schwartz MH, Laskowski PR, Rojek AE, Katanski CD, Riback JA, Dion MF, Franks AM et al (2015) Reversible, specific, active aggregates of endogenous proteins assemble upon heat stress. Cell 162:1286–1298

Westerheide SD, Anckar J, Stevens SM, Sistonen L, Morimoto RI (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science 323:1063–1066

Whitesell L, Lindquist S (2009) Inhibiting the transcription factor HSF1 as an anticancer strategy. Expert Opin Ther Targets 13:469–478

Yamamoto A, Ueda J, Yamamoto N, Hashikawa N, Sakurai H (2007) Role of heat shock transcription factor in Saccharomyces cerevisiae oxidative stress response. Eukaryot Cell 6:1373–1379

Yang S, Huang S, Gaertig MA, Li X-J, Li S (2014) Age-dependent decrease in chaperone activity impairs MANF expression, leading to purkinje cell degeneration in inducible SCA17 mice. Neuron 81:349–365

Zheng X, Krakowiak J, Patel N, Beyzavi A, Ezike J, Khalil AS, Pincus D (2016) Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. Elife 5:e18638

Zheng X, Beyzavi A, Krakowiak J, Patel N, Khalil AS, Pincus D (2018) Hsf1 phosphorylation generates cell-to-cell variation in Hsp90 levels and promotes phenotypic plasticity. Cell Rep 22:3099–3106

Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94:471–480

Part II

Function

4 Challenging Proteostasis: Role of the Chaperone Network to Control Aggregation-Prone Proteins in Human Disease

Tessa Sinnige, Anan Yu, and Richard I. Morimoto

Abstract

Protein homeostasis (Proteostasis) is essential for correct and efficient protein function within the living cell. Among the critical components of the Proteostasis Network (PN) are molecular chaperones that serve widely in protein biogenesis under physiological conditions, and prevent protein misfolding and aggregation enhanced by conditions of cellular stress. For Alzheimer's, Parkinson's, Huntington's diseases and ALS, multiple classes of molecular chaperones interact with the highly aggregation-prone proteins amyloid-β, tau, α-synuclein, huntingtin and SOD1 to influence the course of proteotoxicity associated with these neurodegenerative diseases. Accordingly, overexpression of molecular chaperones and induction of the heat shock response have been shown to be protective in a wide range of animal models of these diseases. In contrast, for cancer cells the upregulation of chaperones has the undesirable effect of promoting cellular survival and tumor growth by stabilizing mutant oncoproteins. In both situations, physiological levels of molecular chaperones eventually become functionally compromised by the persistence of misfolded substrates, leading to a decline in global protein homeostasis and the dysregulation of diverse cellular pathways. The phenomenon of chaperone competition may underlie the broad pathology observed in aging and neurodegenerative diseases, and restoration of physiological protein homeostasis may be a suitable therapeutic avenue for neurodegeneration as well as for cancer.

Keywords

Protein misfolding · Molecular chaperones · Neurodegenerative diseases · Proteostasis

4.1 Introduction

Protein homeostasis is regulated by the proteostasis network (PN) to control protein synthesis, folding and macromolecular assembly, localization, and degradation, processes that are essential for all living cells and organisms. An imbalance in the PN enhances the properties of destabilized mutant proteins that take advantage of the capacity of molecular chaperones to escape unfolding and degradation, leading to malignant phenotypes in cancer (see other chapters in this collection). The opposite scenario of failure of protein homeostasis is associated with aging and a plethora of

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_4

protein misfolding diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). In this chapter, we discuss how interactions between molecular chaperones and neurodegenerative disease-associated substrates amyloid-β (Aβ), tau, α-synuclein, polyglutamine-expansion proteins and SOD1 are exploited *in vivo* to counteract toxic protein aggregation, whereas these same interactions can lead to the sequestration of molecular chaperones and the collapse of proteostasis.

The main classes of molecular chaperones are also known as heat shock proteins (Hsps) after the discovery in *Drosophila* that the expression of Hsps is induced by heat shock, a sudden increase in temperature. However, many of the genes encoding these chaperones are also constitutively expressed to ensure the proper balance of protein synthesis, folding, trafficking and translocation of a wide variety of proteins under physiological conditions. Under conditions of cellular stress such as heat shock, molecular chaperones such as Hsp70 and the J-domain protein Hdj-1 are titrated by misfolded client proteins from association with heat shock transcription factor 1 (HSF1) with which they interact under normal growth conditions (Zheng et al. 2016; Abravaya et al. 1992; Shi et al. 1998). Upon release from chaperones, HSF1 forms functional trimers that bind to heat shock elements in the promoters of genes encoding molecular chaperones and other components of the PN. This results in the release of the paused RNA polymerase II, post-translational modifications of HSF1 and rapid inducible transcription of the heat shock genes. The heat shock transcriptional response can attenuate either during prolonged exposure to heat shock stress as translation is arrested at the heat shock temperature, or upon return to ambient conditions, in both scenarios reducing the requirement for chaperones to prevent further misfolding (Abravaya et al. 1992; Shi et al. 1998; Krakowiak et al. 2018).

Among the most ubiquitous chaperones are the Hsp70 family that is essential for protein synthesis and folding of a wide range of client proteins, via a mechanism that involves cycles of substrate binding and release driven by ATP hydrolysis. Hsp70 and its constitutively expressed counterpart Hsc70 function in a molecular machine that involves co-chaperones including Hsp40 proteins, which recruit substrates and regulate ATP hydrolysis of Hsp70, and Hsp110 and BAG proteins, which serve as nucleotide exchange factors (NEFs) (Kampinga and Craig 2010; Kim et al. 2013). Depending on the nucleotide binding and co-chaperone interactions, Hsp70 can function to hold non-native clients in a folding competent state or direct folding to a functional state (Freeman et al. 1995; Freeman and Morimoto 1996). Furthermore, specific combinations of Hsp70, Hsp40 and Hsp110 proteins form disaggregase machineries that can resolve luciferase aggregates (Nillegoda et al. 2015) and α-synuclein fibrils (Gao et al. 2015). The Hsp70 chaperone system also interacts with Hsp90, which is another ATP-dependent chaperone. Hsp90 interacts with specific co-chaperones downstream of Hsp70 to aid the folding of a wide array of clients including kinases, phosphatases, transcription factors and other signalling molecules (Morán Luengo et al. 2019). The chaperonins of the Hsp60 family, corresponding to GroEL in bacteria and TRiC/CCT in eukaryotes, exist as multimeric assemblies that form a cage in which substrates are allowed to obtain their native fold dependent upon ATP hydrolysis. This machinery is critical to fold large filamentous proteins such as actin and tubulin (Hayer-Hartl et al. 2016; Gestaut et al. 2019). The small Hsps (sHsps) are ATP-independent chaperones and can function in holding denatured or non-native protein conformations to prevent their misfolding and aggregation (Treweek et al. 2015).

In this chapter we will discuss the detailed modes of interaction between these classes of molecular chaperones and aggregation-prone proteins and peptides as characterised *in vitro*, and summarize evidence that chaperone activity is beneficial to combat protein aggregation in *in vivo* models of neurodegenerative diseases. Under these disease conditions, molecular chaperones may eventually become overwhelmed by these misfolding-prone proteins, leading to impaired protein homeostasis of physiological

processes in the cell. The concept of chaperone competition not only serves as an explanation for the collapse of protein homeostasis during aging and neurodegenerative diseases, but also provides opportunities for therapeutic intervention. These approaches are also relevant to cancer in which it has been shown that the downregulation of certain molecular chaperones can be exploited to induce chaperone competition, promoting aggregation or degradation of oncoproteins and halting further proliferation of tumor cells (see other chapters in this collection).

4.2 Molecular Chaperones and Protein Aggregation in Neurodegenerative Diseases

4.2.1 Amyloid-β

Aβ is the main component of extracellular plaques that accumulate in the brains of AD patients. Processing of the amyloid precursor protein (APP) generates multiple isoforms of the Aβ peptide of which the most abundant species, the 42-residue Aβ peptide (Aβ42) is more aggregation-prone than the 40-residue form. *In vitro*, the aggregation of Aβ42 is delayed by the intracellular chaperones Hsp70 and Hsp90 at sub-stoichiometric ratios of ~1:50 (Evans et al. 2006). This effect is dependent on ATP, and the potency of Hsp70 is increased by the co-chaperone Hsp40 (family member DNAJB1), which stimulates ATPase activity, suggesting that to prevent aggregation the chaperones employ the same catalytic cycle that aids protein folding (Evans et al. 2006). Suppression of Aβ42 aggregation can also be achieved by sub-stoichiometric levels of DNAJB6, a member of the Hsp40 family, and quantitative analysis has revealed that DNAJB6 blocks both primary and secondary nucleation (Fig. 4.1) by preferentially binding to oligomeric species (Månsson et al. 2014a). The protective effects of Hsp70 against Aβ-associated toxicity have also been observed *in vivo* in the fruit fly *Drosophila melanogaster*. Overexpression of Hsp70 effectively suppresses neurotoxicity associated with the extracellular deposition of Aβ42 in the fly disease model, irrespective of whether the chaperone is expressed intracellularly or targeted to the extracellular space (Fernandez-Funez et al. 2016; Martín-Peña et al. 2018). The beneficial effect of intracellular Hsp70 may occur by a general enhancement of global proteostasis, whereas in the extracellular space it likely depends on its 'holding' activity in the absence of ATP.

Another class of chaperone activities is represented by the Brichos domains of ProSP-C and Bri2 that inhibit Aβ42 aggregation *in vitro*. Whereas the Brichos domain of ProSP-C blocks secondary nucleation of Aβ42 by binding to the fibrillar surface (Cohen et al. 2015), the Brichos domain derived from Bri2 prevents elongation of Aβ42 in addition to secondary nucleation by binding both to the fibril surface and to fibril ends (Arosio et al. 2016) (Fig. 4.1). Brichos domains physiologically function in the extracellular space, and expression of Brichos domains from ProSP-C and from Bri2 has been shown to be protective in *Drosophila* Aβ42 models by improving motor function and lifespan (Hermansson et al. 2014; Poska et al. 2016). Overexpression of the Brichos domain of ProSP-C increases the levels of soluble versus insoluble Aβ42 and delays its deposition during aging of the flies, suggesting that this Brichos domain could act by preventing the formation of toxic oligomeric species (Hermansson et al. 2014). In further support, ProSP-C Brichos rescued the toxicity of a mixture of Aβ42 monomers and fibrils on mouse brain slices using an electrophysiology assay (Cohen et al. 2015).

A role for the TRiC/CCT chaperonin on Aβ42 phenotypes was demonstrated in a genetic screen of the chaperome in *C. elegans* expressing Aβ42 in the body wall muscle cells. Individual knock-down of each of the eight subunits of TRiC/CCT decreased the motility of the worms, and the screen also identified Hsc70, Hsp40, Hsp90 and its co-chaperones Cdc37 and Sti1 (Brehme et al. 2014). Many of the same chaperones were shown to be protective against toxicity in a *C. elegans* model expressing expanded polyglutamine, suggesting that this subset of

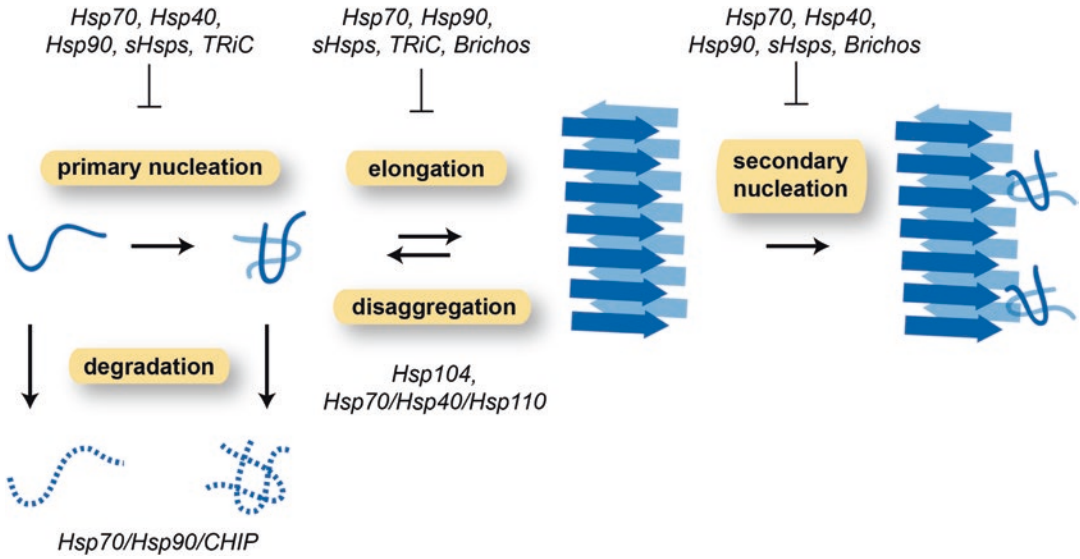

Fig. 4.1 Schematic reaction of amyloid formation indicating where chaperones act to prevent protein misfolding in the case of amyloid-β, tau, α-synuclein, polyglutamine expansion proteins and/or SOD1. Inhibition of primary nucleation is inferred from the binding of chaperones to the monomeric proteins. Hsp90 alone and with its co-chaperone Aha1 has also been reported to promote aggregation in the case of tau

chaperones may have a general beneficial effect on proteostasis, rather than or in addition to making direct interactions with Aβ42. The relevance of TRiC/CCT was furthermore underlined by several of its subunits being downregulated in the aging human brain and in patients with AD (Brehme et al. 2014).

sHsps, a class of ATP-independent chaperones, have also been linked to Aβ aggregation, as it was shown that expression of human Aβ in *C. elegans* body wall muscle cells induces Hsp16 (Link et al. 1999), the overexpression of which completely restores the paralysis phenotype of the Aβ worms (Fonte et al. 2008). This effect appears to result from a direct interaction that modulates aggregation, not only because Hsp16 co-localises with the deposits, but also because its overexpression reduces the amyloid plaque load in the worms, leaving total Aβ levels unaltered. *In vitro*, the sHsp αB-crystallin binds along the sides and at the ends of Aβ42 fibrils, suggesting it can inhibit secondary nucleation and elongation of the fibrils (Shammas et al. 2011).

An important aspect of chaperone biology is that not all chaperones are protective against protein aggregation. The extracellular chaperone clusterin, which is a risk factor for late-onset AD, was reported to have more complex effects in mouse models of Aβ aggregation, presumably because it shifts the clearance pathways of Aβ, thus complicating the interpretation of its potential anti-aggregation effect (DeMattos et al. 2002; Wojtas et al. 2017). Extracellular chaperones may also alter Aβ toxicity by modulating targets that interact with toxic Aβ species. Sti1, which is a co-chaperone of Hsp90, but observed to be secreted by astrocytes, was shown to bind to the PrP^C receptor and thereby block its interaction with Aβ oligomers *in vitro* and in cell culture (Ostapchenko et al. 2013). Increased levels of Sti1 have been observed in aged AD mice, as well as in human AD patients compared to control brains, consistent with a protective mechanism (Ostapchenko et al. 2013).

4.2.2 Tau

Tau is a microtubule-associated protein that forms intracellular aggregates in AD brains, and in patients suffering from various types of frontotemporal dementia (FTD) and ALS, collec-

tively known as Tauopathies. Both Hsp70 and Hsp90 chaperones have been shown to directly interact with tau *in vitro*, which may seem surprising given the disordered and highly hydrophilic nature of tau. However, the sequence motifs that have β-strand propensity, and are involved in the formation of cross-β fibrils, contain hydrophobic residues that mediate binding to the constitutively expressed Hsc70 chaperone (Mitul et al. 2008). Hsp70 also interacts with tau monomers and oligomers to inhibit its nucleation as well as elongation in *in vitro* aggregation assays (Kundel et al. 2018). In *C. elegans* models expressing human tau in mechanosensory neurons, co-expression of human Hsp70 has modest beneficial effects on restoration of the touch response (Miyasaka et al. 2005). In mice, overexpression of Hsp70 reduces endogenous tau levels in aged animals, and especially reduces the insoluble high-molecular weight species (Petrucelli et al. 2004). Similar results have been observed using small molecule inhibitors of the ATPase activity of Hsp70 that lead to reduced levels of total and phosphorylated tau in tau transgenic mice. This suggests that both overexpression and inhibition of the folding cycle of Hsp70 may converge to promote tau degradation by the ubiquitin-proteasome system (Jinwal et al. 2009). Both Hsc70/Hsp70 and Hsp90 are co-localized with tau tangles in a transgenic mouse model and in human AD brains; moreover upregulation of these chaperones suppresses the formation of tau aggregation in cellular models by partitioning tau into a productive folding pathway that restores tau binding to microtubules (Luo et al. 2007).

Studies on Hsp90 have shown that the site of Hsp90 binding on tau includes a broad region encompassing the hydrophobic motifs, generating an extended interaction surface held together by a combination of weak hydrophobic and electrostatic interactions (Karagöz et al. 2014). The levels of phosphorylated tau but not total tau levels in a transgenic mouse model are reduced when the ATPase activity of Hsp90 is inhibited by small molecules (Dickey et al. 2007). Hsp90 inhibition also leads to activation of the heat shock response and the subsequent increase in expression of chaperones including Hsp70, but drug treatment may have a stronger effect than only inducing the heat shock response. Reduction in phospho-tau levels upon the inhibition of Hsp90 may depend on increased activity of its co-chaperone CHIP, which mediates ubiquitination and proteasomal degradation (Dickey et al. 2007). Another small molecule inhibitor of Hsp90 also reduces levels of phosphorylated tau as well as total tau, while increasing Hsp70 levels in mouse models expressing wild-type tau or the FTD-associated tau mutant P301L (Luo et al. 2007). In these studies, Hsp90 interacts directly with the mutant but not wild-type tau, suggesting that the mechanisms promoting tau clearance may differ between the two models, and thus potentially between AD and other types of (familial) dementia (Luo et al. 2007).

Modulation of Hsp90 activity involves co-chaperones such as Aha1, and together these can promote tau aggregation both *in vitro* and in a tau transgenic mouse model. Moreover, a small molecule that blocks the Hsp90-Aha1 interaction reduces this effect, demonstrating the power of combining mechanistic insights from *in vitro* experiments with *in vivo* models in developing therapeutic avenues (Shelton et al. 2017). Co-chaperones may also affect protein aggregation independently, as noted above for Aβ. For tau, it has been shown that the Hsp40 protein DnaJA2 is a potent inhibitor of its aggregation *in vitro (*Mok et al. 2018*)*. DnaJA2 binds to monomeric tau, and also reduces seeded aggregation in cells, suggesting that DnaJA2 may have an effect on multiple steps of the aggregation process. In AD patient neurons with tau pathology, DnaJA2 is highly abundant and is localised with aggregated tau, perhaps by being upregulated as a protective, but insufficient cellular response (Mok et al. 2018). In contrast, FKBP51, another co-chaperone of Hsp90 that co-localises with tau pathology in AD brains may have a role in promoting tau misfolding (Blair et al. 2013). Overexpression of FKBP51 in tau transgenic mice results in increased overall tau levels and neuronal loss, whereas the numbers of tau tangles are decreased. Consistent with these results, *in vitro* experiments have suggested that FKBP51 in complex with Hsp90 may especially increase the

formation of oligomeric tau species (Blair et al. 2013).

A role for the sHsp Hsp27 in modulating tau was shown in transgenic mice overexpressing Hsp27 that reduced tau levels and ameliorated the defects in long-term potentiation. This effect was shown to depend on the oligomerisation of Hsp27, since a phosphorylation mutant of Hsp27 that cannot undergo this cycle did not affect tau pathology or other phenotypes of the mouse (Abisambra et al. 2010).

4.2.3 α-Synuclein

The 140-residue, largely disordered protein α-synuclein is found in intracellular inclusions termed Lewy Bodies, which form primarily in dopaminergic neurons of the substantia nigra in PD patients. Several Hsps have been identified as components of Lewy Bodies (Auluck et al. 2002; McLean et al. 2002; Outeiro et al. 2006), and sub-stoichiometric concentrations of Hsp70 are sufficient *in vitro* to suppress the formation of α-synuclein fibrils in the absence of ATP (Dedmon et al. 2005; Luk et al. 2008; Roodveldt et al. 2009; Aprile et al. 2017). This effect is dependent on the interaction of Hsp70 with the hydrophobic NAC region of α-synuclein, which is essential for fibril formation (Luk et al. 2008). Upon addition of ATP, Hsp70 delays fibril formation primarily by binding to the fibril ends, thus inhibiting elongation (Aprile et al. 2017). Furthermore, a complex of the constitutively expressed Hsc70 together with specific Hsp40 co-chaperones and a Hsp110 NEF can dissociate preformed α-synuclein fibrils (Gao et al. 2015). The effect of Hsp70 on α-synuclein aggregation *in vivo* is thus likely to depend on the relative levels of specific co-chaperones and ATP.

Similarly, in yeast expressing human α-synuclein, induction of Hsp70 expression by a brief heat shock is protective against α-synuclein-induced apoptosis and the generation of reactive oxygen species. Similar effects have been obtained by direct overexpression of the yeast Hsp70 orthologue Ssa3 or by inhibiting Hsp90 using geldanamycin, which also induces the heat shock response (Flower et al. 2005). On the other hand, in *C. elegans*, knock-down of Hsp70 does not affect α-synuclein inclusion formation in muscle cells, suggesting that this chaperone does not have a beneficial effect in this model system (Van Ham et al. 2008). However, knock-down of Hip, an Hsp70 co-chaperone, significantly increases the number of inclusions in this *C. elegans* model, suggesting that the Hsp70-Hip complex acts against inclusion formation (Roodveldt et al. 2009).

In a *Drosophila* model of α-synuclein, co-expression of human Hsp70 with wild-type α-synuclein or the familial mutants A53T or A30P in dopaminergic neurons restores locomotion and lifespan without affecting the number or size of Lewy Body-like inclusions (Auluck et al. 2002). This is further supported by geldanamycin treatment which induces the heat shock response and similarly protects against neurodegeneration (Auluck and Bonini 2002; Auluck et al. 2005), whereas LB-like pathology is not affected and the levels of insoluble α-synuclein are even increased (Auluck et al. 2005), suggesting that Hsp70 may reduce the presence of toxic oligomeric species.

Rodent models for expression of human α-synuclein have yielded inconsistent results. Overexpression of rat Hsp70 in a mouse model resulted in a strong decrease in both high molecular weight α-synuclein species, and Triton X-100 insoluble protein (Klucken et al. 2004), which contrasts with another study in which human Hsp70 and α-synuclein A53T were co-overexpressed and the levels of high molecular weight and insoluble α-synuclein were unaffected (Shimshek et al. 2010). Another study found beneficial effects from co-expressing Hsp70 with α-synuclein in the rat brain, showing a reduction in the number of dystrophic neurites which typically precede neurodegeneration (Moloney et al. 2014). The protective effects of Hsp70 may depend on the ratio of Hsp70, its co-chaperones and α-synuclein in these models as mentioned above, and potential differences in the binding affinities between Hsp70 from different

species and wild-type and A53T α-synuclein could furthermore affect the outcome.

In addition to Hsp70 and Hsp40, the expression of Hsp27 is increased upon viral expression of α-synuclein in mouse brains (St Martin et al. 2007). Likewise, mice expressing α-synuclein A53T had increased levels of Hsp25 and αB-crystallin, Hsp25 being primarily increased in astrocytes rather than neurons. αB-crystallin inhibits *in vitro* aggregation of α-synuclein isolated from the mouse brain (Wang et al. 2008), which is consistent with another *in vitro* result that αB-crystallin interacts directly with α-synuclein fibrils to prevent fibril elongation from pre-formed seeds (Waudby et al. 2010). It has also been reported to interact with early intermediates in *in vitro* aggregation reactions (Rekas et al. 2007). In line with these findings, expression of αB-crystallin in the fly eye ameliorates the rough eye phenotype induced by α-synuclein expression (Tue et al. 2012).

Hsp90 can inhibit α-synuclein aggregation *in vitro* in seeded aggregation assays of α-synuclein A53T. This activity is ATP-independent, and relies on the interaction of Hsp90 with oligomeric α-synuclein species (Daturpalli et al. 2013). The yeast disaggregase Hsp104 can inhibit α-synuclein aggregation and remodel pre-formed α-synuclein fibrils *in vitro*, and overexpressing it together with the A30P α-synuclein mutant in rats reduces inclusion formation and neuronal loss (Lo Bianco et al. 2008).

4.2.4 Polyglutamine Expansions

Nine human neurodegenerative diseases are associated with genetic expansions leading to the production of different proteins with expanded polyglutamine (polyQ) tracts. Irrespective of the protein, disease symptoms occur beyond a pathogenic threshold of ~35–40 glutamine residues, accompanied by the formation of cytoplasmic and nuclear inclusions in neuronal tissue (Lieberman et al. 2019). The onset of pathology and disease is correlated with the length of the polyQ tract, with longer polyQ lengths having increasing aggregation propensity *in vitro* and in cellular models. HD is the most prevalent of these polyQ disorders, and is related to an expansion within the huntingtin gene *HTT*. In particular, a fragment of the huntingtin protein corresponding to the first exon of the gene in which the polyQ expansion is located is found to accumulate in disease, and this fragment is sufficient to drive neurodegeneration and inclusion formation in mouse models (Mangiarini et al. 1996; Scherzinger et al. 1997).

In the nematode worm *C. elegans*, expression of a construct comprising 40 glutamine residues (Q40) with a YFP-tag for visualisation in the body wall muscle cells is sufficient for protein aggregation and formation of toxic immobile inclusions (Morley et al. 2002). Expression of 35 residues (Q35) also leads to aggregation and toxicity, but later in adulthood, whereas shorter polyQ lengths of 19 or 24 glutamine residues remain diffuse. These polyQ lines were used for a genome-wide genetic screen to identify genetic modifiers of protein aggregation which identified components of the proteostasis network for transcription and splicing, translation, folding, transport and degradation including the chaperones Hsp70, Hsp40 and subunits of TRiC/CCT (Nollen et al. 2004). The mechanism by which TRiC/CCT inhibits polyQ aggregation has been further explored *in vitro*, and it was shown to interact with the tips of mutant huntingtin fibrils and to encapsulate smaller oligomers (Shahmoradian et al. 2013).

Other genetic screens in yeast and *Drosophila* have additionally identified multiple Hsps, including Hsp70, Hsp40, Hsp90 and sHsps, as well as Hsp104, the yeast disaggregase (Krobitsch and Lindquist 2000; Willingham et al. 2003; Kazemi-Esfarjani and Benzer 2000; Giorgini et al. 2005; Zhang et al. 2010; Jimenez-Sanchez et al. 2015). Overexpression of Hsp70 and Hsp40 ameliorates multiple phenotypes in *Drosophila* and mouse polyQ disease models, typically without altering the numbers of mature protein aggregates (Warrick et al. 1999; Chan et al. 2000;

Cummings et al. 2001; Hay et al. 2004; Labbadia et al. 2012). Consistent with these findings, Hsp70 has been shown to associate with huntingtin oligomers *in vitro*, but not with monomers or detergent-insoluble inclusions, and it is able to prevent further aggregation together with Hsp40 and in the presence of ATP (Lotz et al. 2010).

More mechanistic studies on the activities of Hsp70, Hsp40 and Hsp110 against huntingtin exon 1 aggregation performed in cell culture have revealed that DNAJB6 and DNAJB8 members of the Hsp40 family are highly effective (Hageman et al. 2010). Subsequently, DNAJB6 was shown to inhibit protein aggregation in an HD mouse model, delaying the onset of symptoms and extending lifespan (Kakkar et al. 2016). *In vitro*, DNAJB6 inhibits the primary nucleation of polyQ peptides which depends on a serine-threonine rich region on its surface (Kakkar et al. 2016). This activity does not, however, depend on the presence of Hsp70 or ATP (Månsson et al. 2014b), providing an interesting example of independent chaperone activity of Hsp40 proteins.

4.2.5 SOD1

Point mutations in superoxide dismutase 1 (SOD1) are one of the causes of familial forms and sporadic cases of ALS (Cook and Petrucelli 2019). In contrast to the proteins discussed above that are largely disordered, SOD1 is a well folded soluble globular protein that binds copper and zinc ions and is stabilized by a disulfide bond. Disease-associated mutations are thought to destabilize the native state of SOD1, rendering it more prone to aggregation (Lindberg et al. 2005; Prudencio et al. 2009).

In a genome-wide RNAi screen on a *C. elegans* strain expressing SOD1 with G85R mutation throughout the neurons, the majority of hits belonged to the category of protein quality control, including the regulator of the heat shock response HSF1, several chaperones and components of the degradation machinery (Wang et al. 2009). Induction of the heat shock response is protective against SOD1 G93A aggregation and toxicity in mice, as demonstrated by the overexpression of SIRT1, which has HSF1 amongst its substrates. The beneficial effect in this case was found to be limited by the expression levels of inducible Hsp70, which were not sufficient to restore the phenotype of mice with higher levels of SOD1 (Watanabe et al. 2014).

A role for the Hsp70/Hsp40/Hsp110 machinery in aggregate disassembly as described above for α-synuclein has also been suggested for SOD1. Overexpression of Hsp110 in mice expressing YFP-tagged SOD1 G85R in motor-neurons extends their lifespan, and a reduction of aggregates has been observed in a subset of animals (Nagy et al. 2016). Addition of YFP-SOD1 G85R to isolated squid axoplasm inhibits axonal transport, and supplementing Hsc70, but more so Hsp110, was found to suppress these defects (Song et al. 2013). Likewise, overexpression of the Hsp40 family member DNAJB2 reduces aggregation of SOD1 G93A in late stages of disease progression in mice, and improves motor-neuron survival and muscle strength. DNAJB2 has been found to associate with the SOD1 aggregates, providing evidence for a direct interaction which was suggested to reduce aggregate formation by promoting ubiquitination (Novoselov et al. 2013).

sHsps have also been proposed to modulate SOD1 aggregation and toxicity. Hsp25 and αB-crystallin co-elute with insoluble SOD1 mutant protein from mice (Wang et al. 2003), and in *in vitro* studies using a brain homogenate, αB-crystallin inhibits aggregation (Wang et al. 2005a). This is further supported by experiments with recombinant Hsp27 and αB-crystallin that inhibit SOD1 G93A aggregation by interfering with aggregate growth, rather than the primary nucleation step (Yerbury et al. 2013). Mutations in Hsp27 have been identified in sporadic cases of ALS, which may be related to the inability to prevent SOD1 aggregation (Capponi et al. 2016).

4.3 Chaperone Competition as a Basis for Proteostasis Collapse in Protein Misfolding Diseases

4.3.1 The Chaperone Competition Hypothesis

The interaction of molecular chaperones with intermediate states of highly aggregation-prone disease-related proteins is a finely tuned process, in which chaperones can either direct and maintain functional client interactions for cellular health or result in protein aggregation and proteome mismanagement in aging and stress. This imbalance can be enhanced by increased protein expression, coding mutations, posttranslational modifications or alterations in the composition and functional properties of the proteostasis network to shift the balance towards aggregation and proteotoxicity. While misfolded and aggregated proteins have been directly linked to cellular toxicity (Bucciantini et al. 2002; Baglioni et al. 2006; Marsh et al. 2000; Fath et al. 2002), the diverse protein misfolding diseases have very complex pathologies likely resulting from multiple molecular defects. The chaperone competition hypothesis provides an explanation why aggregation of a disease-associated protein can interfere with multiple cellular pathways (Yu et al. 2014; Yu et al. 2019). The multifaceted roles of chaperone networks raise the possibility that competition between misfolding proteins and endogenous clients for limited chaperone resources will have consequences on protein functionality. The higher localized concentration of misfolded proteins in aggregates results in a spatial redistribution of chaperones and other components of the proteostasis network. Kinetically, this will have negative effects on many chaperone-regulated processes resulting in multiple pathological symptoms, exacerbating disease progression. Chaperone sequestration initiated by intracellular accumulation of misfolded and aggregated proteins is common to all protein misfolding diseases, supporting the hypothesis that the loss of chaperone function upon protein aggregation can accelerate cellular toxicity.

4.3.2 Chaperone Sequestration During Protein Misfolding

Multiple families of chaperones and co-chaperones form extensive protein-protein interaction networks to assist in the folding of diverse clients. Chaperone-client interactions are transient in nature to allow reversible engagement with multiple substrates including nascent polypeptides, unfolded and misfolded proteins and folding intermediates (Kim et al. 2013; Hiller 2019; Koldewey et al. 2017). Compared with on-pathway substrates for Hsc70, misfolded species are more likely to expose hydrophobic regions to allow Hsc70 to bind with higher apparent avidity (Kundel et al. 2018; Pemberton et al. 2011). Consequently, Hsc70 is preferentially occupied by aberrantly folded protein substrates in stressed cells or upon expression of metastable proteins. When protein aggregates form after stable interaction of misfolded proteins, Hsc70 as well as other interacting partners become sequestered into the aggregates (Fig. 4.2).

Histochemical and biochemical studies have revealed that Hsc70 and other chaperones colocalize with a variety of protein inclusions in multiple cell and animal models of disease-associated protein aggregation and in patient-obtained brain tissues. Proteomic analysis of laser dissected amyloid plaques (Liao et al. 2004) and tau tangles (Wang et al. 2005b) from AD patient brains show that these inclusions sequester molecular chaperones, and other proteins that may be conformationally challenged. Hsc70 and the proteasome also co-localize with intracellular Aβ aggregates in a cellular model (Bückig et al. 2002). A human cell system for seeding-dependent tau aggregation has shown that the chaperones Hsc70/Hsp70, Hsp90 and J-domain co-chaperones are sequestered by tau aggregates (Yu et al. 2019). Likewise in PD, immunohistochemistry and proteomics have identified major chaperones including Hsc70, Hsp90, Hsp40 and

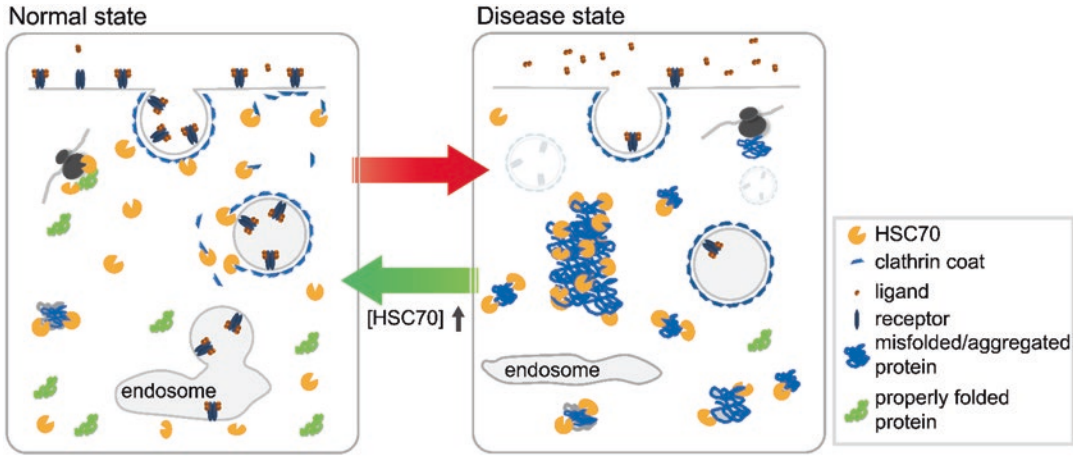

Fig. 4.2 Aggregate-driven chaperone competition explains the pathological complexity associated with disease-associated aggregation-prone proteins. Shown is a model depicting chaperone competition between protein aggregates and the protein folding and vesicular trafficking arms of the proteostasis network. Under normal conditions (left), Hsc70 is at sufficiently high levels to mediate CME as well as basal protein client folding. Under disease conditions where protein aggregates have accumulated and the Hsc70 relocalizes to aggregates, both protein folding and CME are inhibited (right). This can be reversed by increasing the levels of Hsc70 by small molecule activation of HSF1 to restore chaperone function

Hsp27 as constituents of filamentous Lewy bodies, co-localizing with α-synuclein (Uryu et al. 2006; Leverenz et al. 2007).

In the context of HD, Hsc70 binds specifically to the N-terminal flanking region of huntingtin exon 1. Using a conditional human cell system and immunofluorescence, the chaperones BiP/GRP78, Hsp70, Hsp40, proteasome subunits and other aggregation-prone proteins were shown to colocalize with the perinuclear inclusions of huntingtin exon 1 with an expanded polyQ (Waelter et al. 2001). Proteomic profiling of HD inclusions revealed widespread sequestration of proteins into the mutant huntingtin inclusion bodies (Hosp et al. 2017). Similarly, chaperones colocalize with ataxin 1 and ataxin 3 polyQ protein inclusions (Cummings et al. 1998; Chai et al. 1999). For ALS, mutant SOD1 is a substrate of interactions with Hsc70/Hsp70, and mice expressing mutant SOD1 form inclusions containing ubiquitin, the proteasome and Hsc70 (Zetterström et al. 2011). Hsc70-positive inclusions have also been detected in sporadic ALS cases (Watanabe et al. 2001). Chaperone association has also been detected in cells expressing an artificial aggregation-prone β-sheet protein (Olzscha et al. 2011). Collectively, these observations provide strong evidence for sequestration of key components of the proteostasis network such as molecular chaperones and constituents of the protein degradation machinery as a unifying feature of protein misfolding diseases. The delicate balance between the levels of available molecular chaperones and client proteins is further underscored by the fact that many types of cancer cells depend on elevated levels of chaperones for their survival, accommodating for the increased demand by destabilized and misfolding-prone oncoproteins (see other chapters in this collection).

4.3.3 Functional Consequence of Chaperone Sequestration

As described above, many cellular processes are affected by the sequestration of chaperones by protein aggregation. The functional consequences of chaperone competition were determined by measuring multiple Hsc70-mediated cellular processes (Yu et al. 2014, 2019). Clathrin-mediated endocytosis (CME), the main entry route of bio-

logical molecules into the cell (Kirchhausen et al. 2014) involves the self-assembly of trimeric clathrin units on the plasma membrane to cause membrane curvature and the formation of coated pits. The released vesicles rapidly lose their clathrin coats in a process catalyzed by Hsc70 together with the co-chaperone auxilin in order to fuse with intracellular endosomes (Massol et al. 2006). Reducing cellular levels of Hsc70 by siRNA inhibits CME, indicating the requirement of Hsc70 for the assembly and disassembly of clathrin-coated vesicles (Yu et al. 2014).

The kinetic correlation between cytosolic Hsc70 concentration and CME therefore provides a highly sensitive functional readout of chaperone competition in the presence of protein aggregation. By monitoring CME in prostate cancer PC-3 cells expressing different aggregation-prone proteins including polyglutamine, huntingtin, ataxin1 and SOD1, a reduction of CME due to the sequestration of Hsc70 by aggregates was observed (Yu et al. 2014). The sensitivity of CME to Hsc70 depletion suggests that chaperone abundance is rate-limiting in cells expressing aggregation-prone proteins. Moreover, suppression of CME by protein aggregation could be reversed by conditionally increasing Hsc70. These effects were also observed in neuronal cells showing that protein aggregation causes dysregulated internalization of the AMPA receptor, a neuron-specific CME cargo.

The observations of chaperone competition extend beyond Hsc70, as other chaperones, including Hsp90, Hsp40 and Hsp27, are also sequestered in tau inclusions. Single-cell analysis of protein folding and CME in a cell model of tau aggregation reveals that both chaperone-dependent activities are impaired by tau aggregation (Yu et al. 2019). These observations are further supported by the finding in yeast that sequestration of Sis1p, a low-abundant Hsp40 homolog chaperone, by polyQ aggregates interferes with nuclear degradation of misfolded proteins and leads to the formation of cytosolic inclusions (Park et al. 2013). Consequently, the decline in chaperone-dependent arms of the proteostasis network will have profound negative effects on the protein quality control capacity of the cell. Besides cytosolic chaperones, various species of misfolded proteins and aggregates interact with and sequester components of the protein degradation machinery. This sequestration will further compromise the protein quality control capacity (Thibaudeau et al. 2018; Guo et al. 2018; Yang et al. 2015; Holmberg et al. 2004).

4.3.4 Restoration of Chaperone Homeostasis as a Therapeutic Strategy

The functional dissection of chaperone competition will lead to a better understanding of the early events of protein aggregation, and may uncover novel strategies to intervene at early stages of protein misfolding diseases. All Hsc70-regulated processes will be negatively affected by a subcellular redistribution of Hsc70 among its clients, resulting in a decline in multiple chaperone-dependent processes leading to multiple pathological symptoms that exacerbate disease progression. Consequently, restoring Hsc70 homeostasis could be an effective strategy to battle age-related protein conformational diseases. The appearance of a misfolded conformational state of tau associated with CME failure can be detected before the appearance of mature tau inclusions and the stable sequestration of Hsc70 (Yu et al. 2019). The timing of these cellular events therefore suggests that the inhibition of CME is an early cellular event of tauopathy and precedes failure of other cellular processes such as chaperone-dependent protein folding. Moreover, both CME and protein folding can be restored by small molecule regulators of HSF1 resulting in expression of cytosolic chaperones (Yu et al. 2019), suggesting that enhancing chaperone expression could have beneficial consequences on chaperone function and cellular health without reversing tau aggregation. Similarly, overexpressing protein sequestrated by polyQ aggregates has been shown to rescue loss of function phenotypes and relieve polyQ dependent toxicity (Park et al. 2013).

4.4 Outlook

Further studies will be required to bridge the gap between the kinetic studies of protein aggregation and their modulation by molecular chaperones *in vitro* and *in vivo* in healthy and disease states. Cellular probes capable of detecting aggregating protein species, especially in the early and oligomeric states, may allow real-time monitoring of chaperone engagement of disease-associated substrates. Furthermore, molecular chaperones do not operate in isolation and the coordination of chaperone networks to maintain all arms of the proteostasis network needs to be further characterized for each of the aggregation-prone proteins in disease-relevant tissues and cell types. The systems approach for proteostasis in neurodegenerative diseases likely will differ from cancer, although both classes of diseases are related by the consequence of aging on the robustness of cell stress responses and the functional capacity of the proteostasis network. The use of primary patient derived cells and live cell-based approaches to measure the cellular state of proteostasis during disease progression and in the context of aging will provide a basis to screen for small molecules that restore cellular proteostasis. Targeting the restoration of proteostasis, in particular chaperone homeostasis, can potentially serve as a major therapeutic avenue to treat many forms of protein misfolding disorders ranging from neurodegenerative diseases to cancer.

Acknowledgements We thank Christopher M. Dobson for critical reading of part of the manuscript. This work was supported by National Institutes of Health (National Institute on Aging), the Daniel F. and Ada L. Rice Foundation to RIM and research grants from Eli Lilly & Co Ltd, Lilly Research Centre and the Tau Consortium of the Rainwater Foundation.

References

Abisambra JF et al (2010) Phosphorylation dynamics regulate Hsp27-mediated rescue of neuronal Plasticity Deficits in Tau Transgenic Mice. J Neurosci 30:15374 LP–15315382

Abravaya K, Myers MP, Murphy SP, Morimoto RI (1992) The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev 6:1153–1164

Aprile FA et al (2017) Inhibition of α-Synuclein Fibril Elongation by Hsp70 Is Governed by a Kinetic Binding Competition between α-Synuclein Species. Biochemistry 56:1177–1180. https://doi.org/10.1021/acs.biochem.6b01178

Arosio P et al (2016) Kinetic analysis reveals the diversity of microscopic mechanisms through which molecular chaperones suppress amyloid formation. Nat Commun 7:10948

Auluck PK, Bonini NM (2002) Pharmacological prevention of Parkinson disease in Drosophila. Nat Med 8:1185–1186

Auluck PK, Chan HYE, Trojanowski JQ, Lee VMY, Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 295:865–868

Auluck PK, Meulener MC, Bonini NM (2005) Mechanisms of suppression of α-synuclein neurotoxicity by geldanamycin in Drosophila. J Biol Chem 280:2873–2878

Baglioni S et al (2006) Prefibrillar amyloid aggregates could be generic toxins in higher organisms. J Neurosci 26:8160–8167

Blair LJ et al (2013) Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. J Clin Invest 123:4158–4169

Brehme M et al (2014) A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep 9:1–16

Bucciantini M et al (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416:507–511

Bückig A, Tikkanen R, Herzog V, Schmitz A (2002) Cytosolic and nuclear aggregation of the amyloid β-peptide following its expression in the endoplasmic reticulum. Histochem Cell Biol 118:353–360

Capponi S et al (2016) Molecular chaperones in the pathogenesis of amyotrophic lateral sclerosis: the role of HSPB1. Hum Mutat 37:1202–1208

Chai Y, Koppenhafer SL, Bonini NM, Paulson HL (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19:10338–10347

Chan HYE, Warrick JM, Gray-Board GL, Paulson HL, Bonini NM (2000) Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in Drosophila. Hum Mol Genet 9:2811–2820

Cohen SIA et al (2015) A molecular chaperone breaks the catalytic cycle that generates toxic Aβ oligomers. Nat Struct Mol Biol 22:207–213

Cook C, Petrucelli L (2019) Genetic convergence brings clarity to the enigmatic red line in ALS. Neuron 101:1057–1069

Cummings CJ et al (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19:148–154

Cummings CJ et al (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum Mol Genet 10:1511–1518

Daturpalli S, Waudby CA, Meehan S, Jackson SE (2013) Hsp90 inhibits α-synuclein aggregation by interacting with soluble oligomers. J Mol Biol 425:4614–4628

Dedmon MM, Christodoulou J, Wilson MR, Dobson CM (2005) Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. J Biol Chem 280:14733–14740

DeMattos RB et al (2002) Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A 99:10843–10848

Dickey CA et al (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest 117:648–658

Evans CG, Wisén S, Gestwicki JE (2006) Heat shock proteins 70 and 90 inhibit early stages of amyloid β-(1-42) aggregation in vitro. J Biol Chem 281:33182–33191

Fath T, Eidenmüller J, Brandt R (2002) Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease. J Neurosci 22:9733 LP–9739741

Fernandez-Funez P et al (2016) Holdase activity of secreted Hsp70 masks amyloid-β42 neurotoxicity in Drosophila. Proc Natl Acad Sci U S A 113(35):E5212–E5221. https://doi.org/10.1073/pnas.1608045113

Flower TR, Chesnokova LS, Froelich CA, Dixon C, Witt SN (2005) Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. J Mol Biol 351:1081–1100

Fonte V et al (2008) Suppression of in vivo beta-amyloid peptide toxicity by overexpression of the HSP-16.2 small chaperone protein. J Biol Chem 283:784–791

Freeman BC, Morimoto RI (1996) The human cytosolic molecular chaperones hsp90, hsp70 (hsc70) and hdj-1 have distinct roles in recognition of a non-native protein and protein refolding. EMBO J 15:2969–2979

Freeman BC, Myers MP, Schumacher R, Morimoto RI (1995) Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. EMBO J 14:2281–2292

Gao X et al (2015) Human Hsp70 disaggregase reverses Parkinson's-linked α-synuclein amyloid fibrils. Mol Cell 59:781–793

Gestaut D, Limatola A, Joachimiak L, Frydman J (2019) The ATP-powered gymnastics of TRiC/CCT: an asymmetric protein folding machine with a symmetric origin story. Curr Opin Struct Biol 55:50–58

Giorgini F, Guidetti P, Nguyen Q, Bennett SC, Muchowski PJ (2005) A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. Nat Genet 37:526–531

Guo Q et al (2018) In situ structure of neuronal C9orf72 poly-GA aggregates reveals proteasome recruitment. Cell 172:696–705

Hageman J et al (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. Mol Cell 37:355–369

Hay DG et al (2004) Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. Hum Mol Genet 13:1389–1405

Hayer-Hartl M, Bracher A, Hartl FU (2016) The GroEL–GroES chaperonin machine: a nano-cage for protein folding. Trends Biochem Sci 41:62–76

Hermansson E et al (2014) The chaperone domain BRICHOS prevents CNS toxicity of amyloid-β peptide in Drosophila melanogaster. Dis Model Mech 7:659–665

Hiller S (2019) Chaperone-bound clients: the importance of being dynamic. Trends Biochem Sci 44:517–527

Holmberg CI, Staniszewski KE, Mensah KN, Matouschek A, Morimoto RI (2004) Inefficient degradation of truncated polyglutamine proteins by the proteasome. EMBO J 23:4307–4318

Hosp F et al (2017) Spatiotemporal proteomic profiling of Huntington's disease inclusions reveals widespread loss of protein function. Cell Rep 21:2291–2303

Jimenez-Sanchez M et al (2015) siRNA screen identifies QPCT as a druggable target for Huntington's disease. Nat Chem Biol 11:347

Jinwal UK et al (2009) Chemical manipulation of Hsp70 ATPase activity regulates tau stability. J Neurosci 29:12079–12088

Kakkar V et al (2016) The S/T-Rich motif in the DNAJB6 chaperone delays polyglutamine aggregation and the onset of disease in a mouse model. Mol Cell 62:272–283

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592

Karagöz GE et al (2014) Hsp90-tau complex reveals molecular basis for specificity in chaperone action. Cell 156:963–974

Kazemi-Esfarjani P, Benzer S (2000) Genetic suppression of polyglutamine toxicity in Drosophila. Science 287:1837–1840

Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Ulrich Hartl F (2013) Molecular chaperone functions in protein folding and proteostasis. Annu Rev Biochem 82:323–355

Kirchhausen T, Owen D, Harrison SC (2014) Molecular structure, function, and dynamics of clathrin-mediated membrane traffic. Cold Spring Harb Perspect Biol 6:a016725

Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ (2004) Hsp70 reduces alpha-synuclein aggregation and toxicity. J Biol Chem 279:25497–25502

Koldewey P, Horowitz S, Bardwell JCA (2017) Chaperone-client interactions: non-specificity engenders multifunctionality. J Biol Chem 292:12010–12017

Krakowiak J et al (2018) Hsf1 and Hsp70 constitute a two-component feedback loop that regulates the yeast heat shock response. Elife 7:e31668

Krobitsch S, Lindquist S (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. Proc Natl Acad Sci U S A 97:1589–1594

Kundel F et al (2018) Hsp70 inhibits the nucleation and elongation of tau and sequesters tau aggregates with high affinity. ACS Chem Biol 13:636–646

Labbadia J et al (2012) Suppression of protein aggregation by chaperone modification of high molecular weight complexes. Brain 135:1180–1196

Leverenz JB et al (2007) Proteomic identification of novel proteins in cortical lewy bodies. Brain Pathol 17:139–145

Liao L et al (2004) Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. J Biol Chem 279:37061–37068

Lieberman AP, Shakkottai VG, Albin RL (2019) Polyglutamine repeats in neurodegenerative diseases. Annu Rev Pathol Mech Dis 14:1–27

Lindberg MJ, Byström R, Boknäs N, Andersen PM, Oliveberg M (2005) Systematically perturbed folding patterns of amyotrophic lateral sclerosis (ALS)-associated SOD1 mutants. Proc Natl Acad Sci U S A 102:9754 LP–9759759

Link CD, Cypser JR, Johnson CJ, Johnson TE (1999) Direct observation of stress response in Caenorhabditis elegans using a reporter transgene. Cell Stress Chaperones 4:235–242

Lo Bianco C et al (2008) Hsp104 antagonizes a-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. J Clin Invest 118:3087–3097

Lotz GP et al (2010) Hsp70 and Hsp40 functionally interact with soluble mutant huntingtin oligomers in a classic ATP-dependent reaction cycle. J Biol Chem 285:38183–38193

Luk KC, Mills IP, Trojanowski JQ, Lee VMY (2008) Interactions between Hsp70 and the hydrophobic core of alpha-synuclein inhibit fibril assembly. Biochemistry 47:12614–12625

Luo W et al (2007) Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. Proc Natl Acad Sci U S A 104:9511–9516

Mangiarini L et al (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506

Månsson C et al (2014a) Interaction of the molecular chaperone DNAJB6 with growing amyloid-beta 42 (Aβ42) aggregates leads to sub-stoichiometric inhibition of amyloid formation. J Biol Chem 289:31066–31076

Månsson C et al (2014b) DNAJB6 is a peptide-binding chaperone which can suppress amyloid fibrillation of polyglutamine peptides at substoichiometric molar ratios. Cell Stress Chaperones 19:227–239

Marsh JL et al (2000) Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in Drosophila. Hum Mol Genet 9:13–25

Martín-Peña A, Rincón-Limas DE, Fernandez-Fúnez P (2018) Engineered Hsp70 chaperones prevent Aβ42-induced memory impairments in a Drosophila model of Alzheimer's disease. Sci Rep 8:1–14

Massol RH, Boll W, Griffin AM, Kirchhausen T (2006) A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. Proc Natl Acad Sci U S A 103:10265–10270

McLean PJ et al (2002) TorsinA and heat shock proteins act as molecular chaperones: suppression of α-synuclein aggregation. J Neurochem 83:846–854

Mitul S, Jeff K, Gloria L (2008) Two motifs within the tau microtubule-binding domain mediate its association with the hsc70 molecular chaperone. J Neurosci Res 86:2763–2773

Miyasaka T et al (2005) Progressive neurodegeneration in C. elegans model of tauopathy. Neurobiol Dis 20:372–383

Mok SA et al (2018) Mapping interactions with the chaperone network reveals factors that protect against tau aggregation. Nat Struct Mol Biol 25:384–393

Moloney TC et al (2014) Heat shock protein 70 reduces α-synuclein-induced predegenerative neuronal dystrophy in the α-synuclein viral gene transfer rat model of Parkinson's disease. CNS Neurosci Ther 20:50–58

Morán Luengo T, Mayer MP, Rüdiger SGD (2019) The Hsp70–Hsp90 chaperone cascade in protein folding. Trends Cell Biol 29:164–177

Morley JF, Brignull HR, Weyers JJ, Morimoto RI (2002) The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in Caenorhabditis elegans. Proc Natl Acad Sci U S A 99:10417–10422

Nagy M, Fenton WA, Li D, Furtak K, Horwich AL (2016) Extended survival of misfolded G85R SOD1-linked ALS mice by transgenic expression of chaperone Hsp110. Proc Natl Acad Sci U S A 113:5424–5428

Nillegoda NB et al (2015) Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature 524:247–251. https://doi.org/10.1038/nature14884

Nollen EAA et al (2004) Genome-wide RNA interference screen identifies previously undescribed regulators of polyglutamine aggregation. Proc Natl Acad Sci U S A 101:6403–6408

Novoselov SS et al (2013) Molecular chaperone mediated late-stage neuroprotection in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. PLoS One 8:e73944

Olzscha H et al (2011) Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144:67–78

Ostapchenko VG et al (2013) The prion protein ligand, stress-inducible phosphoprotein 1, regulates amyloid-β oligomer toxicity. J Neurosci 33:16552–16564

Outeiro TF et al (2006) Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. Biochem Biophys Res Commun 351:631–638

Park S-H et al (2013) PolyQ proteins interfere with nuclear degradation of cytosolic proteins by sequestering the Sis1p chaperone. Cell 154:134–145

Pemberton S et al (2011) Hsc70 protein interaction with soluble and fibrillar alpha-synuclein. J Biol Chem 286:34690–34699

Petrucelli L et al (2004) CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum Mol Genet 13:703–714

Poska H et al (2016) Dementia-related Bri2 BRICHOS is a versatile molecular chaperone that efficiently inhibits Aβ 42 toxicity in Drosophila. Biochem J 473:3683–3704

Prudencio M, Hart PJ, Borchelt DR, Andersen PM (2009) Variation in aggregation propensities among ALS-associated variants of SOD1: correlation to human disease. Hum Mol Genet 18:3217–3226

Rekas A, Jankova L, Thorn DC, Cappai R, Carver JA (2007) Monitoring the prevention of amyloid fibril formation by α-crystallin. FEBS J 274:6290–6304

Roodveldt C et al (2009) Chaperone proteostasis in Parkinson's disease: stabilization of the Hsp70/alpha-synuclein complex by Hip. EMBO J 28:3758–3770

Scherzinger E et al (1997) Huntingtin encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell 90:549–558

Shahmoradian SH et al (2013) TRiC's tricks inhibit huntingtin aggregation. Elife 2:e00710

Shammas SL et al (2011) Binding of the molecular chaperone αB-crystallin to Aβ amyloid fibrils inhibits fibril elongation. Biophys J 101:1681–1689

Shelton LB et al (2017) Hsp90 activator Aha1 drives production of pathological tau aggregates. Proc Natl Acad Sci U S A 114:201707039

Shi Y, Mosser DD, Morimoto RI (1998) Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev 12:654–666

Shimshek DR, Mueller M, Wiessner C, Schweizer T, van der Putten PH (2010) The HSP70 molecular chaperone is not beneficial in a mouse model of α-synucleinopathy. PLoS One 5:1–7

Song Y et al (2013) Molecular chaperone Hsp110 rescues a vesicle transport defect produced by an ALS-associated mutant SOD1 protein in squid axoplasm. Proc Natl Acad Sci U S A 110:5428 LP–5425433

St Martin JL et al (2007) Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. J Neurochem 100:1449–1457

Thibaudeau TA, Anderson RT, Smith DM (2018) A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. Nat Commun 9:1097

Treweek TM, Meehan S, Ecroyd H, Carver JA (2015) Small heat-shock proteins: important players in regulating cellular proteostasis. Cell Mol Life Sci 72:429–451

Tue NT, Shimaji K, Tanaka N, Yamaguchi M (2012) Effect of αB-crystallin on protein aggregation in Drosophila. J Biomed Biotechnol 2012:1–7

Uryu K et al (2006) Convergence of heat shock protein 90 with ubiquitin in filamentous α-synuclein inclusions of α-synucleinopathies. Am J Pathol 168:947–961

Van Ham TJ et al (2008) C. elegans model identifies genetic modifiers of α-synuclein inclusion formation during aging. PLoS Genet 4:e1000027

Waelter S et al (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol Biol Cell 12:1393–1407

Wang J et al (2003) Copper-binding-site-null SOD1 causes ALS in transgenic mice: aggregates of non-native SOD1 delineate a common feature. Hum Mol Genet 12:2753–2764

Wang J et al (2005a) Somatodendritic accumulation of misfolded SOD1-L126Z in motor neurons mediates degeneration: αB-crystallin modulates aggregation. Hum Mol Genet 14:2335–2347

Wang Q et al (2005b) Proteomic analysis of neurofibrillary tangles in Alzheimer disease identifies GAPDH as a detergent-insoluble paired helical filament tau binding protein. FASEB J 19:869–871

Wang J, Martin E, Gonzales V, Borchelt DR, Lee MK (2008) Differential regulation of small heat shock proteins in transgenic mouse models of neurodegenerative diseases. Neurobiol Aging 29:586–597

Wang J et al (2009) An ALS-linked mutant SOD1 produces a locomotor defect associated with aggregation and synaptic dysfunction when expressed in neurons of Caenorhabditis elegans. PLoS Genet 5:e1000350

Warrick JM et al (1999) Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. Nat Genet 23:425–428

Watanabe M et al (2001) Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. Neurobiol Dis 8:933–941

Watanabe S et al (2014) SIRT1 overexpression ameliorates a mouse model of SOD1-linked amyotrophic lateral sclerosis via HSF1/HSP70i chaperone system. Mol Brain 7:62

Waudby CA et al (2010) The interaction of alphaB-crystallin with mature alpha-synuclein amyloid fibrils inhibits their elongation. Biophys J 98:843–851

Willingham S, Outeiro TF, Devit MJ, Lindquist SL, Muchowski PJ (2003) Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. Science 302:1769–1773

Wojtas AM et al (2017) Loss of clusterin shifts amyloid deposition to the cerebrovasculature via disruption of perivascular drainage pathways. Proc Natl Acad Sci U S A 114:E6962–E6971

Yang H et al (2015) Aggregation of polyglutamine-expanded ataxin 7 protein specifically sequesters ubiquitin-specific protease 22 and deteriorates its deubiquitinating function in the Spt-Ada-Gcn5-Acetyltransferase (SAGA) complex. J Biol Chem 290:21996–22004

Yerbury JJ et al (2013) The small heat shock proteins αb-crystallin and Hsp27 suppress SOD1 aggregation in vitro. Cell Stress Chaperones 18:251–257

Yu A et al (2014) Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. Proc Natl Acad Sci U S A 111:E1481–E1490

Yu A et al (2019) Tau protein aggregates inhibit the protein-folding and vesicular trafficking arms of the cellular proteostasis network. J Biol Chem 294:7917–7930. https://doi.org/10.1074/jbc.RA119.007527

Zetterström P, Graffmo KS, Andersen PM, Brännström T, Marklund SL (2011) Proteins that bind to misfolded mutant superoxide dismutase-1 in spinal cords from transgenic amyotrophic lateral sclerosis (ALS) model mice. J Biol Chem 286:20130–20136

Zhang S, Binari R, Zhou R, Perrimon N (2010) A genome-wide RNA interference screen for modifiers of aggregates formation by mutant Huntingtin in Drosophila. Genetics 184:1165 LP–1161179

Zheng X et al (2016) Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. Elife 5:1–26

The Multifaceted Role of HSF1 in Tumorigenesis

5

Milad J. Alasady and Marc L. Mendillo

Abstract

Heat Shock Factor 1 (HSF1), the master transcriptional regulator of the heat shock response (HSR), was first cloned more than 30 years ago. Most early research interrogating the role that HSF1 plays in biology focused on its cytoprotective functions, as a factor that promotes the survival of organisms by protecting against the proteotoxicity associated with neurodegeneration and other pathological conditions. However, recent studies have revealed a deleterious role of HSF1, as a factor that is co-opted by cancer cells to promote their own survival to the detriment of the organism. In cancer, HSF1 operates in a multifaceted manner to promote oncogenic transformation, proliferation, metastatic dissemination, and anti-cancer drug resistance. Here we review our current understanding of HSF1 activation and function in malignant progression and discuss the potential for HSF1 inhibition as a novel anticancer strategy. Collectively, this ever-growing body of work points to a prominent role of HSF1 in nearly every aspect of carcinogenesis.

Keywords

HSF1 · Chaperones · Cancer · Heat shock · Stress response · Gene regulatory networks · Transcription factor · Heat shock proteins · Tumor

5.1 Introduction

The heat shock response (HSR) is an adaptive mechanism found in all of cellular life that functions to maintain the health of the proteome in times of elevated temperature and other forms of proteotoxic stress (Lindquist 1986). The first report of the HSR, nearly 60 years ago, is a story of serendipitous scientific discovery (Ritossa 1962). A malfunctioning incubator containing drosophila salivary glands overheats and reveals a new pattern of polytene chromosomal puffs– an established marker of active transcription. Within 10 years, it became clear that this phenomenon was associated with a rapid and robust induction of specific transcripts. Because this transcriptional response could be controlled temporally by simply changing temperature, the HSR became a

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_5

powerful and widely utilized system to study gene regulation (Guertin et al. 2010; Teves and Henikoff 2013). Almost immediately upon proteotoxic insult, the cell redirects its gene expression machinery to transcribe and translate heat shock proteins (HSPs), which function as molecular chaperones that serve to maintain proteins in their folded and functional state. Over the years, the HSR has enabled the discovery of numerous fundamental principles of transcription regulation and continues to be used for this purpose today.

While the power of the HSR as a laboratory tool to study gene regulation has been undeniable, a major question remained: what is the role of the HSR in biology? We now recognize the importance of the HSR and HSPs in nearly all aspects of health and disease. In humans, defects in components of the HSR are associated with neurodegenerative diseases, inflammatory diseases, and numerous rare diseases driven by the misfolding and loss of function of individual mutant proteins (Labbadia and Morimoto 2015; Roth and Balch 2011). Tumors, on the other hand, have an exquisite dependence on the HSR. This is most directly evident in the mutated oncoproteins themselves, where the mutations that yield an increase in activity come at the cost of an increased dependence on molecular chaperones (Calderwood and Gong 2016; Calderwood et al. 2006; Jolly and Morimoto 2000). Thus, the role of chaperones in cancer has an extensive history and still remains a topic of great interest.

Just over 10 years ago, two seminal studies provided the most direct evidence for the dependence of cancers on the HSR by asking the following question (Dai et al. 2007; Min et al. 2007). If targeting individual chaperones holds promise as an anti-cancer therapy, what happens if the entire HSR is targeted? To do this, mouse models deficient in Heat Shock Factor 1 (HSF1), the master regulator of the HSR were employed. These initial studies revealed the critical role of HSF1 in enabling tumor formation. The 10 years of research thereafter have been filled with discovery and important mechanistic insights, confirming a fundamental role for this factor in cancer biology. In this chapter, we will discuss our current understanding of the multitude of mechanisms by which HSF1 is activated and enables tumorigenesis by promoting cancer cell survival, proliferation, invasion, and metastasis.

5.2 HSF1 Structure and Function

HSF1 is the canonical member of the highly conserved HSF family of winged alpha helix transcription factors. In mammals, HSF1 contains five domains. The N-terminus contains the DNA-binding domain (DBD). This is followed by the oligomerization domain, which contains leucine zipper repeats 1–3 (LZ1-3; also referred to as heptad repeats HR-A and HR-B), the regulatory domain (RD), and a fourth leucine zipper repeat (LZ4 or HR-C) domain. Lastly, the C-terminus contains the transactivation domain, which interacts with the general transcription machinery to promote the release of promoter-proximal paused RNA Pol II and drive transcription elongation (Anckar and Sistonen 2011; Neudegger et al. 2016; Vihervaara et al. 2017).

The DBD binds to DNA sequences called heat shock elements (HSE). HSEs are alternating inverted repeats of the sequence [nGAAn]. The C-terminus of the DBD wraps around the DNA and exposes the winged domain of HSF1. Thus, in contrast to other winged helix transcription factors, such as the ETS family of transcription factors with winged domains that directly bind DNA (Buchwalter et al. 2004), the winged domain of HSF1 does not make contact with DNA (Gomez-Pastor et al. 2018). Rather, the surface of the winged domain remains exposed for protein-protein interactions and post-translational modifications, both of which can affect HSF1 activity. For example, in the absence of stress, EP300 acetylates HSF1 at K80, ablating the positive charge (Westerheide et al. 2009). This in turn reduces the affinity of HSF1 for DNA (Gomez-Pastor et al. 2018; Jaeger and Whitesell 2018).

The LZ1-3 domain forms intermolecular hydrophobic interactions to mediate HSF1 oligomerization. The LZ1-3 domain can also form an intramolecular interaction with the LZ4 domain via hydrophobic and ionic contacts (Rabindran et al. 1993; Zuo et al. 1994). This

intramolecular interaction inhibits HSF1 oligomerization by sequestering the LZ1-3 domain, preventing it from forming intermolecular interactions with the LZ1-3 domain of other HSF molecules. In mammalian cells, the oligomerization state of HSF1 is clearly important for its activity. Monomeric HSF1 does not bind DNA and is thus inactive as a transcription factor (Gomez-Pastor et al. 2018). The molecular mechanisms that govern these intrinsic conformational changes in response to stress are not well understood.

While a number of models have been proposed to explain HSF1 activation, a consensus is emerging around the chaperone titration model (Abravaya et al. 1992; Shi et al. 1998; Zheng et al. 2016a). In this model, HSF1 is normally bound by chaperones in the cytoplasm. Upon proteotoxic stress, an increased number of misfolded protein substrates compete with HSF1 for chaperone binding, unleashing active HSF1 to drive gene expression. Active HSF1 promotes the transcription of chaperone genes to restore protein homeostasis. Once proteostasis is restored, chaperones are free to inactivate HSF1, completing the negative feedback loop (Gomez-Pastor et al. 2018; Kijima et al. 2018; Shi et al. 1998).

5.2.1 HSF1 Regulation by Post Translational Modification

The RD of HSF1 has long been known to undergo global hyperphosphorylation upon thermal stress that involves the simultaneous phosphorylation of at least 15 serine and threonine residues. While heat-induced global phosphorylation has been used as a marker for HSF1 activation, a series of recent studies demonstrated that this event is largely uncoupled from its transcriptional activity (Budzyński et al. 2015; Zheng et al. 2016b). Specifically, Budzynski et al. generated an HSF1 variant in which 15 S/T phosphorylation sites were simultaneously mutated to alanine within the RD. Surprisingly, this HSF1 mutant was still able to localize to the nucleus, bind HSEs, and increase HSP gene expression in response to acute proteotoxic stress (Budzyński et al. 2015). A subsequent study in yeast went a step further, in which 152 of all 153 serine/threonine residues of yHSF1 were simultaneously mutated to either alanine or aspartate (Zheng et al. 2016b). Remarkably, both of these variants were still functional and capable of driving gene expression during the HSR. These studies did reveal some differences between yeast and human HSF1– the phosphorylation-deficient mutant of human HSF1 moderately increases heat shock-induced transcriptional activity while the phosphorylation-deficient mutant of yeast HSF1 moderately reduces heat shock-induced transcriptional activity. Regardless, these studies collectively demonstrate that global phosphorylation is not necessary for HSF1 function in response to acute proteotoxic stress, but rather acts to fine-tune the transcriptional activity of HSF1 (Budzyński et al. 2015; Zheng et al. 2016b).

While clearly not required for heat shock induction, it is possible that phosphorylation of HSF1 enables it to sense and respond to the physiological stresses that accompany anabolic metabolism, biomass expansion, cellular proliferation, and other cell state fluctuations that occur in normal physiology and disease. These phosphorylation events, driven by diverse signaling pathways, can both promote and inhibit HSF1 activation. The phosphorylation events that positively regulate HSF1 include those on S230 and S320 mediated by calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA), respectively (Holmberg et al. 2001; Zhang et al. 2011). They also include phosphorylation of S326, which is often used as a marker of HSF1 activation even though it is not required for its activity (Chou et al. 2012; Guettouche et al. 2005). On the other hand, phosphorylation events that negatively regulate HSF1 activity include S121 by proinflammatory protein kinase MAPKAP kinase 2 (MK2) (Wang et al. 2006), S363 by c-Jun NH2-terminal kinase (JNK) (Dai et al. 2000), as well as S303 and S307 (Chu et al. 1998; Wang et al. 2003).

Other post-translational modifications (PTMs) in the regulatory domain, such as acetylation and SUMOylation, have also been identified that regulate HSF1. Moreover, there is evidence of

crosstalk between individual PTMs that ultimately impact HSF1 activation. The most prominent example of this is an acetylation-sumoylation switch at K298 that is sensitive to the phosphorylation status of the neighboring S303. Acetylation of K298 prevents proteasome-dependent degradation and therefore increases HSF1 stability (Raychaudhuri et al. 2014). On the other hand, sumoylation of K298, which requires phosphorylation of S303, inhibits HSF1 activity (Hietakangas et al. 2003). These residues are contained within a bipartite ψKxExxSP motif, named PDSM (phosphorylation-dependent sumoylation motif), comprising a SUMO consensus site and a proline-directed phosphorylation site (Hietakangas et al. 2006). PDSM is highly conserved and found in numerous proteins, most notably other transcription regulators, including GATA-1, MEF2, and PPARγ (Hietakangas et al. 2006; Yang and Grégoire 2006). Taken together, there does not seem to be any individual PTM that is sufficient to dramatically alter HSF1 activity– at least in response to thermal stress. Rather, the collective effect of these modifications is to tune HSF1 activity (Anckar and Sistonen 2011). There is still much left to discover with regard to the nature of this PTM combinatorial code, and the precise effects on HSF1 activation, especially in the context of disease-relevant states.

5.3 HSF1 in Carcinogenesis

Two landmark publications provided the first compelling evidence for a direct role of HSF1 in malignancy. In the first study, Hsf1 loss selectively suppressed the formation of lymphomas in a p53-deficient mouse model (Min et al. 2007). In the second study, Hsf1 loss dramatically reduced the susceptibility to tumor formation driven by oncogenic Ras in a classical chemical skin carcinogenesis mouse model, and by a tumor suppressor p53 hotspot mutation (Dai et al. 2007). In this study, Hsf1 knockout mice had reduced numbers and volumes of tumors, and an increase in tumor-free survival (Dai et al. 2007). These initial studies highlight HSF1 as a prominent example of non-oncogene addiction and provide a rationale for targeting HSF1 as an anti-cancer strategy–exploiting the "addiction" of tumors to this evolutionarily conserved survival mechanism (Luo et al. 2009).

The role of HSF1 as a critical pro-tumorigenic factor has been corroborated in a number of additional murine models of cancer. These include a malignant peripheral neural sheath tumor (MPNST) model driven by p53 and Nf1 loss (Dai et al. 2012), a mammary tumorigenesis model driven by Her2/Neu overexpression (Xi et al. 2012), a Hepatocellular Carcinoma (HCC) mouse model driven by chemical carcinogenesis (Jin et al. 2011), and a T-cell Acute Leukemia (T-ALL) model driven by oncogenic Notch1 (Kourtis et al. 2018). In all cases, Hsf1 deletion resulted in a profound reduction in tumor burden, and a corresponding increase in survival of the host.

In humans, elevated nuclear expression of HSF1 is common across diverse types of cancer. These include carcinomas of the breast, cervix, colon, liver, lung, pancreas, and prostate (Dudeja et al. 2011; Fang et al. 2012; Mendillo et al. 2012; Santagata et al. 2011). In addition, these also include mesenchymal tumors such as meningioma (Mendillo et al. 2012), and hematopoietic malignancies such as multiple myeloma and T-ALL (Heimberger et al. 2013; Kourtis et al. 2018).

While HSF1 is expressed in diverse types of cancers, its expression within tumors of any individual type of cancer is heterogeneous. For example, nearly half of breast tumor samples from 1841 women who participated in the Nurses' Health Study have elevated and uniform levels of nuclear HSF1 protein expression (Santagata et al. 2011). Around 30% of the samples have either weak or heterogenous nuclear HSF1 expression. The remaining 20% of the samples are negative for nuclear HSF1 expression. In this study, HSF1 overexpression and nuclear localization were strongly associated with reduced patient survival. These general trends–heterogeneity of HSF1 expression in tumors within a subtype, with high expressing tumors exhibiting more aggressive clinical behavior–are found in many other types of tumors (Engerud et al. 2014; Fang et al. 2012; Liang

et al. 2017; Liao et al. 2015; Mendillo et al. 2012; Wan et al. 2018; Zhou et al. 2017). Taken together, these studies using mouse models and human tumor specimens demonstrate the extraordinary breadth and importance of HSF1 activation across a diverse spectrum of cancers.

5.4 How Is HSF1 Activated in Cancer?

The mechanisms by which HSF1 is activated in cancers appear to be as diverse as the tumor types in which it operates. HSF1, long known for its ability to respond to diverse proteotoxic insults, is clearly well situated to respond to many of the stresses that arise during tumorigenesis. These include cell-autonomous proteotoxic stresses, such as those that accompany increased rates of protein synthesis (Santagata et al. 2013), aneuploidy (Oromendia et al. 2012; Santagata et al. 2013), and misfolded proteins arising from genetic mutations. These also include non-cell-autonomous stresses, such as those that accompany hypoxia, altered nutrient availability, and inflammation (Luo et al. 2009). In these scenarios, the mechanistic basis of HSF1 activation is similar to that which occurs during the heat shock response, i.e. HSF1 is released from normal sequestration by chaperones either by an increase in unfolded substrates or by an increase in protein synthesis and its requisite HSP70-dependent co-translational processing (Fig. 5.1).

Recent work has revealed a multitude of mechanisms by which tumors can seemingly circumvent the requirement of elevated proteotoxic stress and activate HSF1 directly by increasing HSF1 expression, nuclear localization, or stability. Several studies demonstrate that oncogenic signaling pathways activated by either oncogene activation or tumor suppressor loss can directly modulate HSF1 activity, lending support for a proactive mode of activation rather than the canonical stress-sensing mode of activation. HER2 (ERBB2), the oncogenic driver of the eponymous subtype of breast cancers (Slamon et al. 1989), promotes HSF1 activation by at least two mechanisms. HER2 drives PI3K/AKT signaling, which in turn promotes the phosphorylation and inactivation of glycogen synthase kinase-3 beta (GSK3B), which ordinarily phosphorylates HSF1 on S303/307 to inhibit its activity (Chu et al. 1996, 1998; Khaleque et al. 2005). Moreover, PI3K/AKT signaling drives mTOR activation, which in turn phosphorylates HSF1 at S326 and enhances HSF1 transcriptional activity (Chou et al. 2012; Schulz et al. 2014). HSF1 activation driven by this HER2-initiated signaling cascade is of critical importance. In HER2-driven breast cancer cell lines, HSF1 promotes proliferation, migration, mammosphere formation, and xenograft tumor formation, while reducing senescence and apoptosis (Carpenter et al. 2015; Meng et al. 2010; Xi et al. 2012). Likewise, in a HER2+ mouse model, HSF1 enables tumor formation, vascularization, and lung metastasis (Gabai et al. 2012; Xi et al. 2012).

The Mitogen Activated Protein Kinase (MAPK) pathway is commonly altered in multiple types of cancers, with activating mutations in RAF or RAS occurring most frequently. Mutations in RAS activate downstream effectors that include MEK, which interacts with and phosphorylates HSF1 at S326 (Fig. 5.1) (Tang et al. 2015). In addition, loss of the tumor suppressor neurofibromatosis type 1 (NF1) can also activate Ras leading to increased HSF1 phosphorylation, trimerization, nuclear localization, and transcriptional activity (Fig. 5.1) (Dai et al. 2012).

The tumor suppressor kinase LKB1 is inactivated in diverse human cancers that include lung cancer, cervical cancer, and melanoma (Zhou et al. 2014). LKB1 normally activates the metabolic stress sensor AMPK, which suppresses HSF1 activity through S121 phosphorylation, preventing its nuclear translocation, DNA-binding, and transcriptional activity. Loss of AMPK results in increased HSF1 activation (Dai et al. 2015), which in pancreatic ductal adenocarcinoma, promotes invasion and migration (Chen et al. 2017). Taken together, mutations in oncogenes and tumor suppressors converge to induce oncogenic signaling networks, which activate HSF1 to enable malignant progression (Fig. 5.1).

Perhaps the most common mechanisms by which HSF1 is activated in cancer are those

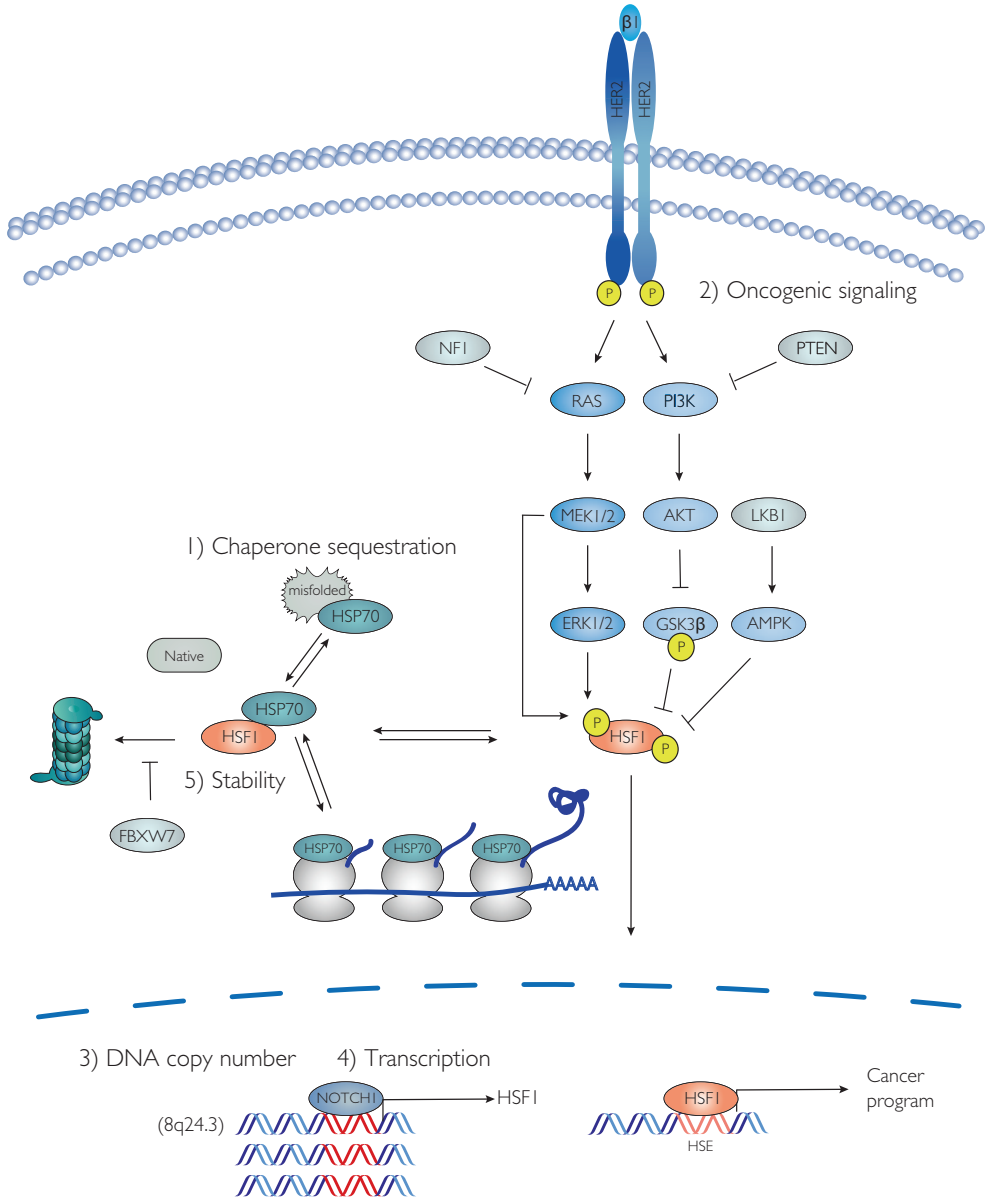

Fig. 5.1 HSF1 activation by multiple mechanisms in cancer. (**1**) Chaperone Sequestration: Under basal conditions, HSF1 remains suppressed by the heat shock protein HSP70. In response to proteotoxic stress or increased levels of protein synthesis, HSF1 is titrated away from HSP70. Subsequently, HSF1 is phosphorylated, trimerizes and translocates to the nucleus. In the nucleus, HSF1 binds to HSEs of target genes and induces gene expression. (**2**) Oncogenic Signaling: Oncogenic signaling pathways activated by either oncogene activation or tumor suppressor loss can regulate HSF1 activity. HER2 drives PI3K/AKT signaling, which in turn promotes the phosphorylation and inactivation of GSK3β. GSK3β phosphorylates HSF1 on S303/307 to inhibit its activity. Activating mutations in RAS activate downstream effectors that include MEK, which interacts with and phosphorylates HSF1 on S326 leading to its activation. Loss of the tumor suppressor neurofibromatosis type 1 (NF1) can also activate RAS leading to increased levels of HSF1 phosphorylation, trimerization, nuclear localization, and transcriptional activation. Loss of tumor suppressor kinase LKB1 inhibits AMPK, which normally inhibits HSF1 via S121 phosphorylation. (**3**) DNA copy number: An increase in HSF1 gene copy number can increase HSF1 mRNA and protein levels. (**4**) mRNA expression levels: NOTCH1 binds directly to the promoter of the HSF1 gene leading to an increase in HSF1 mRNA and protein levels. (**5**) Protein stability: The F-box/WD repeat-containing protein 7 (FBXW7) is an E3 ubiquitin ligase that targets HSF1 for ubiquitylation and proteasomal degradation. Loss of FBXW7 in many cancers leads to an increase in HSF1 stability

which simply increase HSF1 expression levels. One mechanism through which this increase occurs is due to an increase in the copy number of the HSF1 gene itself. (Fig. 5.1). The HSF1 locus resides on chromosomal segment 8q24.3 of the human genome, which is among the most frequently amplified regions across all human cancers (Zhang et al. 2017). HSF1 amplification is likely the mechanistic basis for the increased HSF1 mRNA and protein levels found in ovarian, breast, and prostate cancers, among others (Powell et al. 2016; Santagata et al. 2011). In other cancers, HSF1 can be upregulated directly at the level of transcription. A recent study demonstrated that the oncogene NOTCH1, which is hyperactivated in T cell acute lymphoblastic leukemia (T-ALL), binds directly to the promoter of HSF1 to drive its transcription (Fig. 5.1). This leads to increased HSF1 protein levels and consequently, increased HSF1-dependent transcription of HSPs. In addition, NOTCH1 also binds directly to many of these same HSP genes to independently drive their expression. This NOTCH1-HSF1-HSP feedforward loop is essential for T-ALL pathogenesis. Loss of HSF1 eradicates leukemia in mouse models of T-ALL, while sparing normal hematopoiesis. Moreover, disruption of this feedforward loop at any node, by depletion of NOTCH1, HSF1 or any of the downstream HSP targets, suppresses the growth of human T-ALL (Kourtis et al. 2018).

HSF1 nuclear protein levels can also be elevated by reducing the rate of HSF1 degradation. The F-box/WD repeat-containing protein 7 (FBXW7) is a component of the multi-subunit ubiquitin ligase (SCF), which functions in the ubiquitin-dependent proteasome degradation pathway. FBXW7 is a well-characterized tumor suppressor associated with multiple cancers, such as carcinomas of the breast, prostate, and pancreas, among others (Akhoondi et al. 2007). A recent study demonstrated that elevated nuclear HSF1 protein levels correlate with loss of FBXW7 in melanoma where HSF1 promotes metastatic and invasive properties. Mechanistically, FBXW7 is an E3 ubiquitin ligase that targets HSF1 for ubiquitylation and proteosomal degradation (Fig. 5.1) (Kourtis et al. 2015). Given that FBXW7 is mutated in many cancers, it is likely this mechanism of increasing the levels of active HSF1 is relevant in other types of cancers. More broadly, FBXW7-dependent degradation of HSF1 may also be a contributing factor in neurodegenerative diseases such as Huntington Disease (Gomez-Pastor et al. 2017).

Importantly, the mechanisms described above are not inconsistent with the canonical chaperone-sequestration model of HSF1 activation. Rather, an increase in HSF1 levels simply reduces the degree to which chaperone sequestration is required to achieve the same level of HSF1 activity. In sum, HSF1 is not only activated in response to oncogenic stresses but is also activated by a number of other mechanisms, such as those mediated by oncogenic signaling and those that simply increase HSF1 expression levels, which collectively explain the breadth of HSF1 activation and function in tumorigenesis.

5.5 How Does HSF1 Support the Malignant State in Cancer?

HSF1 regulates a transcriptional network of classical HSPs and a wide array of other genes directly involved in many of the hallmark processes of cancer, including cancer cell proliferation, invasion, and energy metabolism (Hanahan and Weinberg 2011). In contrast to HSF1-dependent transcription during heat shock (Mendillo et al. 2012) or viral infection (Filone et al. 2014), where the net effect of HSF1 activity is a profound induction of chaperones (e.g. HSF1-dependent steady-state mRNA levels of some HSP70s are increased hundreds of fold upon heat shock), HSF1-dependent transcription in cancer results in a more nuanced effect on transcription. In this scenario, HSF1 tunes the expression of targets that support a diverse array of biological processes. It is worth noting that the HSP and non-HSP target genes most sensitive to HSF1 activity have a moderate reduction (~two-fold) in steady-state mRNA levels after HSF1 depletion in whole population experiments. It is

likely that there are subpopulations of cells where HSF1 has a more profound effect on transcription, and these cells might be particularly important in promoting aggressive cancer phenotypes and drug resistance. Lastly, a subset of genes that are bound by HSF1 directly have increased expression upon HSF1 depletion in cancer cells (Mendillo et al. 2012). One possible explanation is that HSF1 can suppress the expression of a subset of its targets through mechanisms that have yet to be defined. However, another possibility is that HSF1 still drives the gene expression of these targets, but HSF1 loss enables a more potent transcription factor to bind and drive gene expression to even higher levels. In sum, HSF1 rewires the cancer cell transcriptome, with implications that are discussed in more detail below.

5.5.1 HSF1 Regulation of Cancer Cell Proteostasis

The HSP genes regulated by HSF1 include HSP70, HSP90 and other chaperones and co-chaperones that are often expressed at elevated levels in cancers. While these chaperones will be covered in more detail in other chapters of this volume, we will briefly discuss how these genes may contribute to the HSF1 cancer program.

HSP70 has a well-established role in promoting survival through its regulation of apoptosis, senescence, and autophagy (Murphy 2013). For example, HSP70 associates with the caspase recruitment domain (CARD) of Apaf1 and inhibits the formation of the apoptosome, which is normally required for activation of pro-caspase 9 (Alnemri et al. 2000; Green et al. 2000). In addition, HSP70 depletion leads to the release of cytochrome c and a decrease in lysosome integrity–the lysosome becomes permeable and releases cathepsin B, a protease that may activate caspases directly (Dudeja et al. 2009). In breast and pancreatic cancer, HSP70 depletion decreases cancer cell growth by significantly inducing cell death (Nylandsted et al. 2002; Phillips et al. 2007). Thus, it is conceivable that HSF1 suppresses cancer cell apoptosis by promoting HSP70 transcription. In support of this idea, Jacobs et al. revealed an HSF1-HSP70/BAG3 axis required to prevent apoptosis in colon cancers. Here, HSP70 and its nucleotide exchange factor BAG3 (Bcl-2 associated athanogene domain 3) interact with the anti-apoptotic mediator BCL2 to prevent its degradation resulting in reduced levels of apoptosis (Jacobs and Marnett 2009). In addition, HSF1, by promoting HSP70 expression, inhibits the phosphorylation and activation of the pro-apoptotic, c-Jun N-terminal kinase (JNK), which is known to induce apoptosis (Jacobs and Marnett 2007). Likewise, in chronic lymphocytic leukemia (CLL), inhibition of HSF1 and HSP70 induces apoptosis in vitro (Åkerfelt et al. 2010; Frezzato et al. 2019). Collectively, these studies demonstrate that HSF1 promotes cancer cell survival at least in part through its regulation of HSP70 (Fig. 5.2).

HSP90 is another chaperone protein with a well-characterized role in malignancy (Whitesell and Lindquist 2005). HSP90 associates with substrates or "client proteins" involved in diverse cellular processes that include signal transduction, immune response, development and DNA repair. Instead of recognizing a specific sequence within a protein, HSP90 is thought to recognize structurally unstable conformations of client proteins (Schopf et al. 2017; Taipale et al. 2012; Taipale et al. 2014). In addition, mutations in these proteins can increase conformational instability that render them more dependent on HSP90 and other chaperones to maintain their proper folding and activity (Sahni et al. 2015). In support of this idea, there is an extensive body of research that demonstrates the importance of HSP90 in chaperoning mutated oncoproteins critical in carcinogenesis (Jaeger and Whitesell 2018; Whitesell and Lindquist 2005). In fact, Geldanamycin and other members of the benzoquinone ansamycin class of HSP90 inhibitors were originally thought to directly inhibit the V-Src oncogene, which reflects the extraordinary dependence of V-Src and other oncogenic tyrosine kinases on HSP90 (Whitesell et al. 1994). Beyond stabilizing essential oncogenic clients, HSP90 may also impact tumorigenesis by promoting the evolution of heritable new traits. A

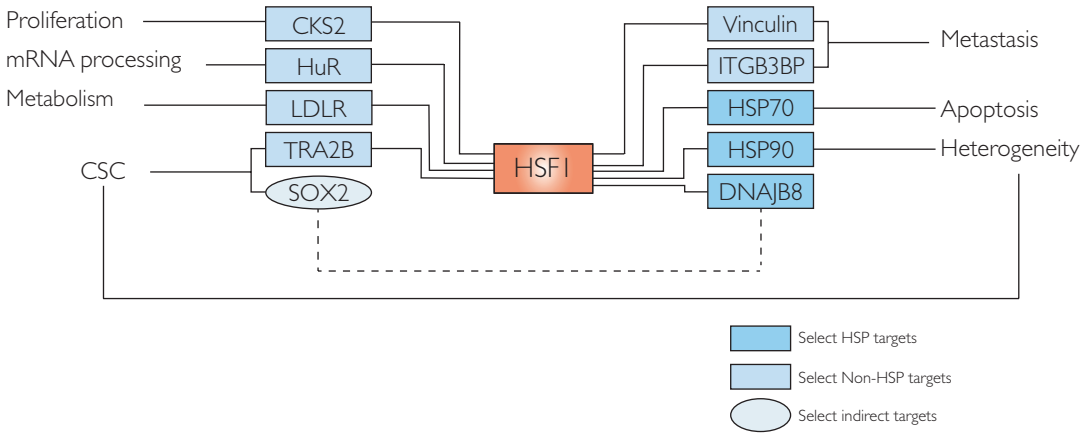

Fig. 5.2 HSF1 targets and their role in malignancy. A subset of the diverse targets of the HSF1 cancer program are depicted. HSF1 activates canonical HSPs such as HSP70 and HSP90 that promote tumorigenesis by multiple mechanisms. For example, HSP70 has an established role as a factor that inhibits apoptosis. HSP90 enables heterogeneity, which promotes aggressive cancer phenotypes. DNAJB8 promotes the conversion of non-cancer stem cells into cancer-stem cells (CSC) by increasing the expression of SOX2. HSF1 also promotes tumorigenesis by regulating the transcription of genes that encode non-HSP factors that are involved in processes that include proliferation, mRNA processing, and metabolism, among others

large body of work in model organisms has established a role for HSP90 in promoting phenotypic robustness by masking the deleterious effects of destabilizing mutations and regulating the folding of a diverse spectrum of signaling proteins (Jarosz and Lindquist 2010; Queitsch et al. 2002; Rutherford and Lindquist 1998). Because of this, it has been described as an "evolutionary capacitor" due to its ability to store phenotypic variance, which can be released upon cellular stress (Jarosz et al. 2010). This HSP90-mediated link between cellular stress and phenotypic diversification is likely to have important implications in human malignancies (Jarosz 2016; Whitesell and Lindquist 2005). In human models of breast cancer, modest HSP90 inhibition, which does not possess anticancer activity on its own, strongly impaired the emergence of resistance to hormone antagonists in cell culture and in mice (Whitesell et al. 2014). In another example, modest HSP90 inhibition exacerbated the chemosensitivity of cells that encode mutant Fanconi anemia pathway proteins (Karras et al. 2017). Thus, by promoting the transcription of HSP90 and other chaperones, HSF1 will likely affect the oncogenic signaling circuitry, heterogeneity and evolvability of human cancers (Fig. 5.2) (Jaeger and Whitesell 2018).

5.5.2 HSF1 Regulation of mRNA Processing and Protein Synthesis

The direct targets regulated by HSF1 in malignancy include genes that are involved in mRNA processing and protein synthesis, processes that are often aberrantly regulated to support the increased levels of cell proliferation associated with malignant progression (Truitt and Ruggero 2016). As one example, HSF1 directly regulates the splicing factor TRA2B (also known as also SFRS10) (Kajita et al. 2013; Mendillo et al. 2012). Mutation analysis of the TRA2B promoter revealed that two of three HSEs are particularly important for the induction of TRA2B in response to oxidative stress (Kajita et al. 2013). In breast, cervical, ovarian, and colon cancer, TRA2B upregulation has been suggested to play a role in metastasis by affecting the splicing of genes involved in proliferation and cell survival (Best et al. 2013). Beyond simply promoting proliferation, TRA2B has been reported to modulate other

processes relevant to tumorigenesis, including lipid metabolism (Pihlajamäki et al. 2011) and developmental gene regulation (Fig. 5.2) (Dichmann et al. 2015).

The RNA-binding protein HuR (ELAVL1) provides another example of an important effector of the HSF1 cancer program that has been particularly well studied (Fig. 5.2). HSF1 binds the promoter of HuR to directly promote its expression (Chou et al. 2015; Gabai et al. 2012; Holmes et al. 2018; Mendillo et al. 2012). In turn, HuR binds an AU-rich consensus motif in the 3′-untranslated region of RNA targets to enhance their translation and/or stability (Srikantan and Gorospe 2012). HuR targets include hypoxia-inducible factor 1 (HIF-1), β-catenin, and Rictor which promote angiogenesis, invasion, stem cell renewal (Chou et al. 2015; Gabai et al. 2012; Holmes et al. 2018). Another HuR target is SIRT1 (Abdelmohsen et al. 2007; Chou et al. 2015; Gabai et al. 2012; Holmes et al. 2018), which can deacetylate HSF1 to increase its activity (Westerheide et al. 2009). This HSF1-HuR-SIRT1 circuit has been shown to promote HSF1 activity in response to DNA-damage-mediated senescence (Kim et al. 2012) and serves as one of several examples of a link between HSF1 and the response to DNA damage, further detailed below.

5.5.3 HSF1 Regulation of DNA Repair

Recent work has revealed a role for HSF1 in managing DNA repair, most prominently in response to genotoxic stress that arises from anti-cancer therapies. Fujimoto et al. report an HSF1-PARP13-PARP1 complex that is required for tumorigenesis. Here, HSF1 recruits PARP1 to chromatin as a ternary complex with PARP13. In response to DNA damage, PARP1 is released from the complex and is redirected to sites of DNA damage to promote DNA repair (Fujimoto et al. 2017). The loss of either HSF1 or PARP13 reduces PARP1 chromatin occupancy and the efficiency of homologous recombination repair (HRR). As an interesting extension of this work, the group went on to show that genotoxic stress that disrupts the HSF1-PARP13-PARP1 complex reduces HSP70 expression during the HSR, suggesting crosstalk between genotoxic and proteotoxic stresses (Fujimoto et al. 2018). Ordinarily, the HSF1-PARP13-PARP1 complex binds to the HSP70 promoter to promote its expression during the HSR. The results of this study show that, in response to thermal stress, PARP1 is redistributed throughout the HSP70 locus resulting in HSP70 PARylation, which is required for HSF1 binding to the HSP70 promoter for optimal HSP70 induction. Further support for a role of HSF1 in maintaining genome integrity is the HSF1-BCL6-TOX axis in germinal center (GC) B cells. HSF1-dependent activation of BCL6 represses the expression of BLC6 targets that include TOX, a DNA binding protein of the HMG-box family that is involved in chromatin assembly, transcription, and replication. As a result of TOX repression, DNA repair mechanisms are enhanced in cancers with high levels of stresses leading to chemoresistance (Fernando et al. 2019). These studies reveal the multifaceted mechanisms by which HSF1 is involved in the maintenance of genome integrity in tumorigenesis.

5.5.4 HSF1 Regulation of Energy Metabolism

HSF1 has a role in regulating cellular metabolism, and thus may play a role in the aberrant metabolism that has long been recognized as a hallmark of malignancy. As one example, HSF1 directly increases the transcription of lactate dehydrogenase A (LDH-A), which increases lactate production, and consequently promotes glycolysis (Dai et al. 2007; Zhao et al. 2009). This HSF1-mediated addiction to glucose can be exploited with the natural product englerin A (EA). EA induces insulin resistance, which deprives tumor cells access to glucose, and simultaneously activates protein kinase C-θ, which activates HSF1, leading to a lethal scenario in highly glycolytic tumors (Sourbier et al. 2013).

In mouse hepatocytes, HSF1 loss reduces the levels of NAD^+, ATP, and glucose resulting in an

increase in AMPK activation, and a reduction in mitochondrial respiration and lipid synthesis (Qiao et al. 2017). Mechanistically, this occurs at least in part because HSF1 directly promotes the transcription of NAMPT, which maintains intracellular NAD+ levels through the NAD^+ salvage pathway. A very recent study has also revealed that HSF1 can inhibit AMPK activity independent of its transcriptional activity through a physical interaction that reduces the affinity of AMPK to AMP (Su et al. 2019). Because AMPK normally limits the activity of the lipogenic transcription factor SREBP1, HSF1 can promote lipogenesis through the expression of SREBP1 target genes that include fatty acid synthase (FASN) and low-density liporprotein receptor (LDLR). Interestingly, HSF1 can also directly regulate the transcription of FASN and LDLR (Mendillo et al. 2012) demonstrating that HSF1 uses both transcriptional and non-transcriptional mechanisms in a feedforward-like manner to regulate lipogenesis.

5.5.5 HSF1 Regulation of Cell Motility, Migration and Adhesion

HSF1 directly regulates the expression of a number of genes involved in cell motility, migration and adhesion, which may be particularly important in promoting cancer cell invasion and metastasis. In one study, overexpression of a constitutively active variant of HSF1 promoted the anchorage-independent growth, migration and metastatic dissemination of melanoma cells by directly suppressing the transcription of Vinculin (Toma-Jonik et al. 2015), a focal adhesion gene previously observed to suppress invasion and metastasis (Goldmann et al. 2013). A different study of the pro-metastatic function of HSF1 in melanoma highlighted ITGB3BP as another example of an HSF1 target gene involved in cell migration. However, this study suggested that HSF1 target genes involved in other processes, such as the proliferation gene CKS2, the metabolic enzyme MTHFD2, and canonical HSPs, among others, are also important (Fig. 5.2). Taken together, these studies provide a plausible mechanistic basis for the identification of HSF1 as one of six metastasis promoting genes in a genome-wide screen for drivers of melanoma invasion as well as the observation that HSF1 is correlated with poor clinical outcomes in malignant melanoma (Scott et al. 2011). More broadly, because HSF1 directly regulates many of these pro-metastatic targets described above in other types of cancer (Mendillo et al. 2012), this same aspect of HSF1 regulation may explain the correlations observed between HSF1 activation and aggressive phenotypes in multiple myeloma, and cancers of the pancreas, prostate, lung and breast (Dudeja et al. 2011; Fang et al. 2012; Mendillo et al. 2012; Nakamura et al. 2014; Santagata et al. 2011; Toma-Jonik et al. 2015).

5.5.6 HSF1 Regulation of Cell State

Finally, HSF1 may promote malignant progression by altering cancer stem cell (CSC)–like characteristics. There is a wealth of data that implicates CSCs in tumor formation, metastatic dissemination, and drug resistance (Scheel and Weinberg 2012). A series of recent studies have reported elevated HSF1 expression in CSC-like cancer cells coincident with an increase in CSC markers that include CD44, SOX2, and Nanog (Kusumoto et al. 2018; Wang et al. 2015; Yasuda et al. 2017). Functionally, HSF1 promotes tumor sphere formation independent of cell proliferation, suggesting that these changes are due to changes in CSC-like characteristics (Wang et al. 2015). Mechanistically, it is possible that HSF1 mediates these effects through canonical regulation of HSPs. In support of this possibility, cellular stresses that include thermal and oxidative stress have been shown to differentiate non-CSC into CSC-like cancer cells. In this mechanism, HSF1 drives the expression of DNAJB8, a member of the HSP 40 family, which in turn is critical for SOX2 upregulation (Kusumoto et al. 2018). However, a non-mutually exclusive possibility is that HSF1 mediates these effects through the direct regulation of either developmental genes such as JARID2 or genes involved in other pro-

cesses, such as the splicing factor TRA2B that has been reported to regulate the expression of the CSC marker CD44 (Fig. 5.2). Future studies will be required to better understand the molecular mechanisms by which the complex network of HSF1 target genes contribute to promote tumorigenesis. Because most HSF1 target genes are also regulated by other factors, experiments that attempt to phenocopy the effects of HSF1 loss by knocking out its target genes are not optimal. Ideally, strategies should be employed that disrupt the regulatory circuitry of HSF1 at subsets of its targets (e.g. targeting the HSE of HSP genes directly) that leave other mechanisms of their regulation intact. While challenging, advances in genome engineering make these types of experiments an exciting possibility.

5.6 Outlook

The association of the HSF1 cancer program with anabolic cellular processes, metastases and death suggests an evolutionary origin distinct from cancer itself (Jaeger and Whitesell 2018; Mendillo et al. 2012). Moreover, the broad range of cancer types in which HSF1 is activated lends further support that this program originated to support basic biological processes (Mendillo et al. 2012; Santagata et al. 2011). Recent work has revealed that the sole heat-shock factor in *C. elegans* drives an essential transcriptional program during development that is distinct from the heat shock response. In this context, the binding of E2F recruits HSF1 to genes containing a distinct consensus sequence comprising a GC-rich motif coupled to a degenerate HSE to drive a transcriptional program essential for *C. elegans* larval development. Thus, certain aspects of HSF1 function in cancer may derive from an ancient role in development that is conserved across species (Li et al. 2016). Related to this, HSF2, one of several HSF1 paralogs, has been reported to function as a stress sensor during development (Åkerfelt et al. 2010). In addition, several recent studies demonstrate that HSF2 also has a role in tumorigenesis (Björk et al. 2016; Zhong et al. 2016). However, in contrast to the pro-tumorigenic HSF1, there is data to suggest that it can both suppress (Björk et al. 2016) and promote tumorigenesis (Zhong et al. 2016), depending on the context. Much remains to be understood regarding the role of HSF2 and potentially other HSFs in cancer, including whether there is an interplay in cancer cells between these HSFs, which can form hetero-oligomers (Alastalo et al. 2003; He et al. 2003; Jaeger et al. 2016).

The multitude of mechanisms by which HSF1 operates to support tumorigenesis make it an attractive target for cancer therapy. However, targeting transcription factors with small molecules is notoriously challenging. Because of this, defining the critical upstream regulatory nodes that feed into HSF1 provides an indirect strategy to identify pharmacologically tractable targets to disrupt the HSF1 cancer program. In fact, most reported inhibitors of HSF1 are the result of phenotypic screens that act through either critical cofactors or nodes upstream of HSF1. In one example, an inhibitor that targets eIF4A (Iwasaki et al. 2016), which is involved in cap recognition during translation initiation, was identified that leads to the inactivation of HSF1 (Santagata et al. 2013). In another example, an inhibitor that targets CDK9, which is involved in transcription elongation, was identified that leads to the inhibition of HSF1 activity during the HSR (Rye et al. 2016).

Several emerging strategies provide hope that we will be able to develop potent and specific inhibitors that directly target HSF1. In one recent example, molecular modeling was used to predict molecules that would bind the HSF1 DNA binding domain. A subsequent screen of candidates led to the development of HSFI115, which was shown to bind the DNA binding domain and inhibit HSF1 transcriptional activity (Vilaboa et al. 2017). In another recent pair of examples, dominant negative peptide screens resulted in the identification of peptides that target the DNA-binding domain, the trimerization domain (Dorrity et al. 2019), and the LZ4 domain of HSF1 (Ran et al. 2018), all of which led to the inhibition of HSF1 activity. Another emerging strategy is PROTAC (Proteolysis-Targeting Chimera), which harnesses the power of the

ubiquitin-proteasome to selectively target and degrade proteins. A PROTAC is a bivalent molecule comprising one domain that selectively binds a target and another domain that binds an E3 ligase, resulting in the ubiquitination and degradation of the target protein (Pettersson and Crews 2019). PROTAC could theoretically be coupled to molecules based on the molecular modeling and peptide screens described above to develop even more potent molecules that not only inactivate, but also degrade HSF1. This is all the more important considering that HSF1 is now appreciated to function in both the tumor cell and the tumor ecosystem (Scherz-Shouval et al. 2014); (discussed in an accompanying chapter) to impact nearly all aspects of tumorigenesis.

Acknowledgements We thank members of the Mendillo laboratory for comments on the manuscript. M.L.M is supported by the National Cancer Institute of the NIH (R00CA175293) and the Susan G. Komen Foundation (CCR17488145). M.L.M was also supported by Kimmel Scholar (SKF-16-135) and Lynn Sage Scholar awards. M.J.A. is supported by the Northwestern University Training Program in Signal Transduction and Cancer (T32 CA070085).

References

Abdelmohsen K et al (2007) Phosphorylation of HuR by Chk2 regulates SIRT1 expression. Mol Cell 25(4):543–557. https://doi.org/10.1016/j.molcel.2007.01.011

Abravaya K et al (1992) The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. Genes Dev 6(7):1153–1164. https://doi.org/10.1101/gad.6.7.1153.

Åkerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol. Nature Publishing Group 11(8):545–555. https://doi.org/10.1038/nrm2938.

Akhoondi S et al (2007) FBXW7/hCDC4 is a general tumor suppressor in human cancer. Cancer Res 67(19):9006–9012. https://doi.org/10.1158/0008-5472.CAN-07-1320

Alastalo T-P et al (2003) Formation of nuclear stress granules involves HSF2 and coincides with the nucleolar localization of Hsp70. J Cell Sci 116(Pt 17):3557–3570. https://doi.org/10.1242/jcs.00671

Alnemri ES et al (2000) Negative regulation of the Apaf-1 apoptosome by Hsp70. Nat Cell Biol 2(8):476–483. https://doi.org/10.1038/35019510

Anckar J, Sistonen L (2011) Regulation of H SF 1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80(1):1089–1115. https://doi.org/10.1146/annurev-biochem-060809-095203

Best A et al (2013) Expression of Tra2 β in cancer cells as a potential contributory factor to neoplasia and metastasis. International J Cell Biol 2013:843781. https://doi.org/10.1155/2013/843781

Björk JK et al (2016) Heat-shock factor 2 is a suppressor of prostate cancer invasion. Oncogene 35(14):1770–1784. https://doi.org/10.1038/onc.2015.241

Buchwalter G, Gross C, Wasylyk B (2004) Ets ternary complex transcription factors. Gene 324(1–2):1–14. https://doi.org/10.1016/j.gene.2003.09.028

Budzyński MA et al (2015) Uncoupling stress-inducible phosphorylation of heat shock factor 1 from its activation. Mol Cell Biol 35(14):2530–2540. https://doi.org/10.1128/mcb.00816-14

Calderwood SK, Gong J (2016) Heat shock proteins promote cancer: it's a protection racket. Trends Biochem Sci 41(4):311–323. https://doi.org/10.1016/j.tibs.2016.01.003

Calderwood SK et al (2006) Heat shock proteins in cancer: chaperones of tumorigenesis. Trends Biochem Sci 31(3):164–172. https://doi.org/10.1016/j.tibs.2006.01.006

Carpenter RL et al (2015) Akt phosphorylates and activates HSF-1 independent of heat shock, leading to slug overexpression and epithelial-mesenchymal transition (EMT) of HER2-overexpressing breast cancer cells. Oncogene. https://doi.org/10.1038/onc.2013.582

Chen K et al (2017) Loss of AMPK activation promotes the invasion and metastasis of pancreatic cancer through an HSF1-dependent pathway. Mol Oncol 11(10):1475–1492. https://doi.org/10.1002/1878-0261.12116

Chou SD et al (2012) mTOR is essential for the proteotoxic stress response, HSF1 activation and heat shock protein synthesis. PLoS One 7(6):1–9. https://doi.org/10.1371/journal.pone.0039679

Chou SD et al (2015) HSF1 regulation of β-catenin in mammary cancer cells through control of HuR/elavL1 expression. Oncogene. Nature Publishing Group 34(17):2178–2188. https://doi.org/10.1038/onc.2014.177.

Chu B et al (1996) Sequential phosphorylation by mitogen-activated protein kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. J Biol Chem. https://doi.org/10.1074/jbc.271.48.30847

Chu B et al (1998) Transcriptional activity of heat shock factor 1 at 37 °c is repressed through phosphorylation on two distinct serine residues by glycogen synthase kinase 3α and protein kinases cα and cζ. J Biol Chem. https://doi.org/10.1074/jbc.273.29.18640

Dai R et al (2000) c-Jun NH2-terminal kinase targeting and phosphorylation of heat shock factor-1 suppress its transcriptional activity. J Biol Chem 275(24):18210–18218. https://doi.org/10.1074/jbc.M000958200

Dai C et al (2007) Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. Cell. https://doi.org/10.1016/j.cell.2007.07.020

Dai C et al (2012) Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. J Clin Investig. https://doi.org/10.1172/JCI62727

Dai S et al (2015) Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. EMBO J 34(3):275–293. https://doi.org/10.15252/embj.201489062

Dichmann DS, Walentek P, Harland RM (2015) The alternative splicing regulator Tra2b is required for somitogenesis and regulates splicing of an inhibitory Wnt11b isoform. Cell Rep 10(4):527–536. https://doi.org/10.1016/j.celrep.2014.12.046

Dorrity MW, Queitsch C, Fields S (2019) High-throughput identification of dominant negative polypeptides in yeast. Nat Methods. https://doi.org/10.1038/s41592-019-0368-0

Dudeja V et al (2009) Heat shock protein 70 inhibits apoptosis in cancer cells through simultaneous and independent mechanisms. Gastroenterology. AGA Institute American Gastroenterological Association 136(5):1772–1782. https://doi.org/10.1053/j.gastro.2009.01.070

Dudeja V et al (2011) Prosurvival role of heat shock factor 1 in the pathogenesis of pancreatobiliary tumors. Am J Physiol Gastrointest Liver Physiol 300(6):G948–G955. https://doi.org/10.1152/ajpgi.00346.2010

Engerud H et al (2014) High level of HSF1 associates with aggressive endometrial carcinoma and suggests potential for HSP90 inhibitors. Br J Cancer. https://doi.org/10.1038/bjc.2014.262

Fang F, Chang R, Yang L (2012) Heat shock factor 1 promotes invasion and metastasis of hepatocellular carcinoma in vitro and in vivo. Cancer 118(7):1782–1794. https://doi.org/10.1002/cncr.26482

Fernando TM et al (2019) BCL6 evolved to enable stress tolerance in vertebrates and is broadly required by cancer cells to adapt to stress. Cancer Discov 9(5):662–679. https://doi.org/10.1158/2159-8290.CD-17-1444

Filone CM et al (2014) The master regulator of the cellular stress response (HSF1) is critical for Orthopoxvirus infection. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1003904

Frezzato F et al (2019) HSP70/HSF1 axis, regulated via a PI3K/AKT pathway, is a druggable target in chronic lymphocytic leukemia. Int J Cancer. https://doi.org/10.1002/ijc.32383

Fujimoto M et al (2017) The HSF1-PARP13-PARP1 complex facilitates DNA repair and promotes mammary tumorigenesis. Nat Commun. Springer US 8(1). https://doi.org/10.1038/s41467-017-01807-7

Fujimoto M et al (2018) Crossm shock response by facilitating heat shock transcription.

Gabai VL et al (2012) Heat shock transcription factor Hsf1 is involved in tumor progression via regulation of hypoxia-inducible factor 1 and RNA-binding protein HuR. Mol Cell Biol 32(5):929–940. https://doi.org/10.1128/mcb.05921-11

Goldmann WH et al (2013) Vinculin, cell mechanics and tumour cell invasion. Cell Biol Int. https://doi.org/10.1002/cbin.10064

Gomez-Pastor R et al (2017) Abnormal degradation of the neuronal stress-protective transcription factor HSF1 in Huntington's disease. Nat Commun. Nature Publishing Group 8(May 2016):1–17. https://doi.org/10.1038/ncomms14405

Gomez-Pastor R, Burchfiel ET, Thiele DJ (2018) Regulation of heat shock transcription factors and their roles in physiology and disease. Nat Rev Mol Cell Biol. Nature Publishing Group 19(1):4–19. https://doi.org/10.1038/nrm.2017.73.

Green DR et al (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2(8):469–475. https://doi.org/10.1038/35019501

Guertin MJ et al (2010) Drosophila heat shock system as a general model to investigate transcriptional regulation. Cold Spring Harb Symp Quant Biol 75:1–9. https://doi.org/10.1101/sqb.2010.75.039

Guettouche T et al (2005) Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. BMC Biochem 6:1–14. https://doi.org/10.1186/1471-2091-6-4.

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell. https://doi.org/10.1016/j.cell.2011.02.013

He H et al (2003) Elevated expression of heat shock factor (HSF) 2A stimulates HSF1-induced transcription during stress. J Biol Chem 278(37):35465–35475. https://doi.org/10.1074/jbc.M304663200.

Heimberger T et al (2013) The heat shock transcription factor 1 as a potential new therapeutic target in multiple myeloma. Br J Haematol 160(4):465–476. https://doi.org/10.1111/bjh.12164

Hietakangas V et al (2003) Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. Mol Cell Biol *23*(8):2953–2968

Hietakangas V et al (2006) PDSM, a motif for phosphorylation-dependent SUMO modification. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.0503698102

Holmberg CI et al (2001) Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. EMBO J 20(14):3800–3810. https://doi.org/10.1093/emboj/20.14.3800

Holmes B et al (2018) MTORC2/AKT/HSF1/HuR constitute a feed-forward loop regulating Rictor expression and tumor growth in glioblastoma. Oncogene 37(6):732–743. https://doi.org/10.1038/onc.2017.360

Iwasaki S, Floor SN, Ingolia NT (2016) Rocaglates convert DEAD-box protein eIF4A into a sequence-selective translational repressor. Nature 534(7608):558–561. https://doi.org/10.1038/nature17978

Jacobs AT, Marnett LJ (2007) Heat shock factor 1 attenuates 4-Hydroxynonenal-mediated apoptosis. J Biol

Chem 282(46):33412–33420. https://doi.org/10.1074/jbc.m706799200

Jacobs AT, Marnett LJ (2009) HSF1-mediated BAG3 expression attenuates apoptosis in 4-hydroxynonenal-treated colon cancer cells via stabilization of anti-apoptotic Bcl-2 proteins. J Biol Chem 284(14):9176–9183. https://doi.org/10.1074/jbc.M808656200

Jaeger AM, Whitesell L (2018) HSP90: enabler of cancer adaptation. Ann Rev Cancer Biol 3(1):275–297. https://doi.org/10.1146/annurev-cancerbio-030518-055533

Jaeger AM et al (2016) Structures of HSF2 reveal mechanisms for differential regulation of human heat-shock factors. Nat Struct Mol Biol 23(2):147–154. https://doi.org/10.1038/nsmb.3150

Jarosz D (2016) Hsp90. A global regulator of the genotype-to-phenotype map in cancers. Adv Cancer Res. https://doi.org/10.1016/bs.acr.2015.11.001

Jarosz DF, Lindquist S (2010) Hsp90 and environmental stress transform the adaptive value of natural genetic variation. Science. https://doi.org/10.1126/science.1195487

Jarosz DF, Taipale M, Lindquist S (2010) Protein homeostasis and the phenotypic manifestation of genetic diversity: principles and mechanisms. Annu Rev Genet 44(1):189–216. https://doi.org/10.1146/annurev.genet.40.110405.090412

Jin X, Moskophidis D, Mivechi NF (2011) Heat shock transcription factor 1 is a key determinant of HCC development by regulating hepatic steatosis and metabolic syndrome. Cell Metab. https://doi.org/10.1016/j.cmet.2011.03.025

Jolly C, Morimoto RI (2000) Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 92(19):1564–1572. https://doi.org/10.1093/jnci/92.19.1564.

Kajita K et al (2013) Ets1 and heat shock factor 1 regulate transcription of the transformer 2β gene in human colon cancer cells. J Gastroenterol 48(11):1222–1233. https://doi.org/10.1007/s00535-012-0745-2

Karras GI et al (2017) HSP90 shapes the consequences of human genetic variation. Cell. https://doi.org/10.1016/j.cell.2017.01.023

Khaleque MA et al (2005) Induction of heat shock proteins by heregulin β1 leads to protection from apoptosis and anchorage-independent growth. Oncogene 24(43):6564–6573. https://doi.org/10.1038/sj.onc.1208798

Kijima T et al (2018) HSP90 inhibitors disrupt a transient HSP90-HSF1 interaction and identify a noncanonical model of HSP90-mediated HSF1 regulation. Sci Rep 8(1):1–13. https://doi.org/10.1038/s41598-018-25404-w

Kim G et al (2012) Maintenance of senescence phenotype. Aging Cell 11(4):617–627. https://doi.org/10.1111/j.1474-9726.2012.00827.x

Kourtis N et al (2015) FBXW7 modulates cellular stress response and metastatic potential through HSF1 post-translational modification. Nat Cell Biol 17(3):322–332. https://doi.org/10.1038/ncb3121

Kourtis N et al (2018) Oncogenic hijacking of the stress response machinery in T cell acute lymphoblastic leukemia. Nat Med. Springer US 24(8):1157–1166. https://doi.org/10.1038/s41591-018-0105-8

Kusumoto H et al (2018) Cellular stress induces cancer stem-like cells through expression of DNAJB8 by activation of heat shock factor 1. Cancer Sci 109(3):741–750. https://doi.org/10.1111/cas.13501

Labbadia J, Morimoto RI (2015) The biology of proteostasis in aging and disease. Annu Rev Biochem 84:435–464. https://doi.org/10.1146/annurev-biochem-060614-033955

Li J et al (2016) E2F coregulates an essential HSF developmental program that is distinct from the heat-shock response. Genes Dev 30(18):2062–2075. https://doi.org/10.1101/gad.283317.116

Liang W et al (2017) Heat shock factor 1 inhibits the mitochondrial apoptosis pathway by regulating second mitochondria-derived activator of caspase to promote pancreatic tumorigenesis. J Exp Clin Cancer Res. https://doi.org/10.1186/s13046-017-0537-x

Liao Y et al (2015) Higher heat shock factor 1 expression in tumor stroma predicts poor prognosis in esophageal squamous cell carcinoma patients. J Transl Med. https://doi.org/10.1186/s12967-015-0703-x

Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191. https://doi.org/10.1146/annurev.bi.55.070186.005443

Luo J, Solimini NL, Elledge SJ (2009) Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 136(5):823–837. https://doi.org/10.1016/j.cell.2009.02.024

Mendillo ML et al (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. Cell 150(3):549–562. https://doi.org/10.1016/j.cell.2012.06.031

Meng L, Gabai VL, Sherman MY (2010) Heat-shock transcription factor HSF1 has a critical role in human epidermal growth factor receptor-2-induced cellular transformation and tumorigenesis. Oncogene. https://doi.org/10.1038/onc.2010.277

Min JN et al (2007) Selective suppression of lymphomas by functional loss of Hsf1 in a p53-deficient mouse model for spontaneous tumors. Oncogene. https://doi.org/10.1038/sj.onc.1210317

Murphy ME (2013) The HSP70 family and cancer. Carcinogenesis 34(6):1181–1188. https://doi.org/10.1093/carcin/bgt111

Nakamura Y et al (2014) Heat shock factor 1 is required for migration and invasion of human melanoma in vitro and in vivo. Cancer Lett. Elsevier Ireland Ltd 354(2):329–335. https://doi.org/10.1016/j.canlet.2014.08.029

Neudegger T et al (2016) Structure of human heat-shock transcription factor 1 in complex with DNA. Nat Struct Mol Biol. Nature Publishing Group 23(2):140–146. https://doi.org/10.1038/nsmb.3149.

Nylandsted J et al (2002) Selective depletion of heat shock protein 70 (Hsp70) activates a tumor-specific death program that is independent of caspases and bypasses Bcl-2. Proc Natl Acad Sci 97(14):7871–7876. https://doi.org/10.1073/pnas.97.14.7871

Oromendia AB, Dodgson SE, Amon A (2012) Aneuploidy causes proteotoxic stress in yeast. Genes Dev. https://doi.org/10.1101/gad.207407.112

Pettersson M, Crews CM (2019) PROteolysis TArgeting chimeras (PROTACs) – past, present and future. Drug Discov Today Technol 31:15–27. https://doi.org/10.1016/j.ddtec.2019.01.002

Phillips PA et al (2007) Triptolide induces pancreatic cancer cell death via inhibition of heat shock protein 70. Cancer Res 67(19):9407–9416. https://doi.org/10.1158/0008-5472.CAN-07-1077

Pihlajamäki J et al (2011) Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. Cell Metab 14(2):208–218. https://doi.org/10.1016/j.cmet.2011.06.007

Powell CD et al (2016) The heat shock transcription factor HSF1 induces ovarian cancer epithelial-mesenchymal transition in a 3D spheroid growth model. PLoS One 11(12):1–16. https://doi.org/10.1371/journal.pone.0168389

Qiao A et al (2017) The transcriptional regulator of the chaperone response HSF1 controls hepatic bioenergetics and protein homeostasis. J Cell Biol 216(3):723–741. https://doi.org/10.1083/jcb.201607091

Queitsch C, Sangstert TA, Lindquist S (2002) Hsp90 as a capacitor of phenotypic variation. Nature. https://doi.org/10.1038/nature749

Rabindran SK et al (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. Science. https://doi.org/10.1126/science.8421783

Ran X et al (2018) Rational design and screening of peptide-based inhibitors of heat shock factor 1 (HSF1). Bioorg Med Chem 26(19):5299–5306. https://doi.org/10.1016/j.bmc.2018.04.018

Raychaudhuri S et al (2014) Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. Cell. https://doi.org/10.1016/j.cell.2014.01.055

Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia. https://doi.org/10.1007/BF02172188

Roth DM, Balch WE (2011) Modeling general proteostasis: proteome balance in health and disease. Curr Opin Cell Biol 23(2):126–134. https://doi.org/10.1016/j.ceb.2010.11.001

Rutherford SL, Lindquist S (1998) Hsp90 as a capacitor for morphological evolution. Nature. https://doi.org/10.1038/24550

Rye CS et al (2016) Discovery of 4,6-disubstituted pyrimidines as potent inhibitors of the heat shock factor 1 (HSF1) stress pathway and CDK9. MedChemComm 7(8):1580–1586. https://doi.org/10.1039/c6md00159a

Sahni N et al (2015) Widespread macromolecular interaction perturbations in human genetic disorders. Cell. https://doi.org/10.1016/j.cell.2015.04.013

Santagata S et al (2011) High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. Proc Natl Acad Sci 108(45):18378–18383. https://doi.org/10.1073/pnas.1115031108

Santagata S et al (2013) Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. Science 341(6143). https://doi.org/10.1126/science.1238303

Scheel C, Weinberg RA (2012) Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. Semin Cancer Biol 22(5–6):396–403. https://doi.org/10.1016/j.semcancer.2012.04.001

Scherz-Shouval R et al (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. Cell 158(3):564–578. https://doi.org/10.1016/j.cell.2014.05.045

Schopf FH, Biebl MM, Buchner J (2017) The HSP90 chaperone machinery. Nat Rev Mol Cell Biol. https://doi.org/10.1038/nrm.2017.20

Schulz R et al (2014) HER2/ErbB2 activates HSF1 and thereby controls HSP90 clients including MIF in HER2-overexpressing breast cancer. Cell Death Dis 5(1):1–11. https://doi.org/10.1038/cddis.2013.508

Scott KL et al (2011) Proinvasion metastasis drivers in early-stage melanoma are oncogenes. Cancer Cell. Elsevier Inc 20(1):92–103. https://doi.org/10.1016/j.ccr.2011.05.025

Shi Y, Mosser DD, Morimoto RI (1998) Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev 12(5):654–666. https://doi.org/10.1101/gad.12.5.654

Slamon DJ et al (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science (New York, NY) 244(4905):707–712. https://doi.org/10.1126/science.2470152.

Sourbier C et al (2013) Englerin a stimulates PKCθ to inhibit insulin Signaling and to simultaneously activate HSF1: pharmacologically induced synthetic lethality. Cancer Cell. Elsevier Inc. 23(2):228–237. https://doi.org/10.1016/j.ccr.2012.12.007

Srikantan S, Gorospe M (2012) HuR function in disease. Frontiers in bioscience (Landmark edition).

Su K-H et al (2019) Heat shock factor 1 is a direct antagonist of AMP-activated protein kinase. Mol Cell. https://doi.org/10.1016/j.molcel.2019.08.021

Taipale M et al (2012) Quantitative analysis of Hsp90-client interactions reveals principles of substrate recognition. Cell. https://doi.org/10.1016/j.cell.2012.06.047

Taipale M et al (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. Cell. Elsevier Inc 158(2):434–448. https://doi.org/10.1016/j.cell.2014.05.039

Tang Z et al (2015) MEK guards proteome stability and inhibits tumor-suppressive amyloidogenesis via HSF1. Cell. Elsevier Inc. 160(4):729–744. https://doi.org/10.1016/j.cell.2015.01.028

Teves SS, Henikoff S (2013) The heat shock response: a case study of chromatin dynamics in gene regulation. Biochem Cell Biol 91(1):42–48. https://doi.org/10.1139/bcb-2012-0075

Toma-Jonik A et al (2015) Active heat shock transcription factor 1 supports migration of the melanoma cells via vinculin down-regulation. Cell Signal. Elsevier B.V. 27(2):394–401. https://doi.org/10.1016/j.cellsig.2014.11.029

Truitt ML, Ruggero D (2016) New frontiers in translational control of the cancer genome. Nat Rev Cancer 16(5):288–304. https://doi.org/10.1038/nrc.2016.27

Vihervaara A et al (2017) Transcriptional response to stress is pre-wired by promoter and enhancer architecture. Nat Commun. https://doi.org/10.1038/s41467-017-00151-0

Vilaboa N et al (2017) New inhibitor targeting human transcription factor HSF1: effects on the heat shock response and tumor cell survival. Nucleic Acids Res 45(10):5797–5817. https://doi.org/10.1093/nar/gkx194

Wan T et al (2018) Prognostic role of HSF1 overexpression in solid tumors: a pooled analysis of 3,159 patients. OncoTargets Ther. https://doi.org/10.2147/OTT.S153682

Wang X et al (2003) Regulation of molecular chaperone gene transcription involves the serine phosphorylation, 14-3-3 epsilon binding, and cytoplasmic sequestration of heat shock factor 1. Mol Cell Biol 23(17):6013–6026. https://doi.org/10.1128/MCB.23.17.6013

Wang XZ et al (2006) Phosphorylation of HSF1 by MAPK-activated protein kinase 2 on serine 121, inhibits transcriptional activity and promotes HSP90 binding. J Biol Chem 281(2):782–791. https://doi.org/10.1074/jbc.M505822200

Wang B et al (2015) Heat shock factor 1 induces cancer stem cell phenotype in breast cancer cell lines. Breast Cancer Res Treat. Springer US 153(1):57–66. https://doi.org/10.1007/s10549-015-3521-1

Westerheide SD et al (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT. Science. https://doi.org/10.1126/science.1165946

Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5(10):761–772. https://doi.org/10.1038/nrc1716

Whitesell L et al (1994) Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. In: Proceedings of the National Academy of Sciences of the United States of America.

Whitesell L et al (2014) HSP90 empowers evolution of resistance to hormonal therapy in human breast cancer models. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.1421323111

Xi C et al (2012) Heat shock factor Hsf1 cooperates with ErbB2 (Her2/Neu) protein to promote mammary tumorigenesis and metastasis. J Biol Chem 287(42):35646–35657. https://doi.org/10.1074/jbc.M112.377481.

Yang XJ, Grégoire S (2006) A recurrent Phospho-Sumoyl switch in transcriptional repression and beyond. Mol Cell. https://doi.org/10.1016/j.molcel.2006.08.009

Yasuda K et al (2017) Phosphorylation of HSF1 at serine 326 residue is related to the maintenance of gynecologic cancer stem cells through expression of HSP27. Oncotarget 8(19):31540–31553. https://doi.org/10.18632/oncotarget.16361

Zhang Y et al (2011) Protein kinase a regulates molecular chaperone transcription and protein aggregation. PLoS One 6(12). https://doi.org/10.1371/journal.pone.0028950

Zhang CQ et al (2017) Overexpressed HSF1 cancer signature genes cluster in human chromosome 8q. Hum Genomics. https://doi.org/10.1186/s40246-017-0131-5

Zhao YH et al (2009) Upregulation of lactate dehydrogenase A by ErbB2 through heat shock factor 1 promotes breast cancer cell glycolysis and growth. Oncogene 28(42):3689–3701. https://doi.org/10.1038/onc.2009.229

Zheng X et al (2016a) Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. elife 5:1–26. https://doi.org/10.7554/elife.18638

Zheng X et al (2016b) Dynamic control of Hsf1 during heat shock by a chaperone switch and phosphorylation. elife 5:3–5. https://doi.org/10.7554/elife.18638

Zhong Y-H et al (2016) Heat shock factor 2 is associated with the occurrence of lung cancer by enhancing the expression of heat shock proteins. Oncol Lett 12(6):5106–5112. https://doi.org/10.3892/ol.2016.5368

Zhou W, Zhang J, Marcus AI (2014) LKB1 tumor suppressor: therapeutic opportunities knock when LKB1 is inactivated. Genes Dis. https://doi.org/10.1016/j.gendis.2014.06.002

Zhou Z et al (2017) Heat shock transcription factor 1 promotes the proliferation, migration and invasion of osteosarcoma cells. Cell Prolif. https://doi.org/10.1111/cpr.12346

Zuo J et al (1994) Activation of the DNA-binding ability of human heat shock transcription factor 1 may involve the transition from an intramolecular to an intermolecular triple-stranded coiled-coil structure. Mol Cell Biol. https://doi.org/10.1128/mcb.14.11.7557

6 Chaperome Networks – Redundancy and Implications for Cancer Treatment

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Abstract

The chaperome is a large family of proteins composed of chaperones, co-chaperones and a multitude of other factors. Elegant studies in yeast and other organisms have paved the road to how we currently understand the complex organization of this large family into protein networks. The goal of this chapter is to provide an overview of chaperome networks in cancer cells, with a focus on two cellular states defined by chaperome network organization. One state characterized by chaperome networks working in isolation and with little overlap, contains global chaperome networks resembling those of normal, non-transformed, cells. We propose that in this state, redundancy in chaperome networks results in a tumor type unamenable for single-agent chaperome therapy. The second state comprises chaperome networks interconnected in response to cellular stress, such as MYC hyperactivation. This is a state where no redundant pathways can be deployed, and is a state of vulnerability, amenable for chaperome therapy. We conclude by proposing a change in how we discover and implement chaperome inhibitor strategies, and suggest an approach to chaperome therapy where the properties of chaperome networks, rather than genetics or client proteins, are used in chaperome inhibitor implementation.

Keywords

Protein network connectivity · Chaperome networks · Protein network vulnerability · Epichaperome · Anti-cancer therapy · HSP90 inhibitors · PU-H71

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6.1 The Chaperome

The term chaperome was introduced in 2006 to denote an assembly of chaperones, co-chaperones and related factors (Wang et al. 2006). An initial list of the human chaperome was published in 2013 and reported 147 bioinformatically predicted members (Finka and Goloubinoff 2013). It

M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_6

included members of the heat shock protein 90 (HSP90)s, HSP70s, HSP60, HSP110s, HSP40s (also known as DNAJ proteins), HSP10, and the small HSPs (sHSPs), as well as their co-chaperones and members of the folding peptidylprolyl isomerase (PPI) and protein disulfide isomerase enzymes. The name of each HSP family is derived from the molecular weight of the original founding member. The name of heat shock proteins (HSPs), has its roots in the discovery of the heat shock response. This arose from the observed puffing pattern in a *Drosophila* chromosome and is a sign of enhanced transcription of genes encoding HSPs (Ritossa 1962, 1964). Ultimately, a conserved group of proteins produced in response to heat and other stresses was identified (Richter et al. 2010). However, it is important to emphasize that HSPs are only a small subset of the chaperome (Finka et al. 2011). In eukaryotes, most families also have organelle-specific members, such as those expressed in the endoplasmic reticulum (ER) and mitochondria (Czarnecka et al. 2006; Lee 2014; Voos and Rottgers 2002). Later studies expanded the chaperome list to 332 chaperones and co-chaperones, represented by 88 chaperones (27%), of which 50 were ATP-dependent, and 244 co-chaperones (73%) (Brehme and Voisine 2016; Brehme et al. 2014; Hadizadeh Esfahani et al. 2018). The chaperome selection was rationalized as a result of a member's involvement in proteotoxic stress (Brehme and Voisine 2016). Several tetratricopeptide repeat (TPR)-domain-containing proteins were also included, based on their functional interactions with select chaperones.

An analysis of protein expression in immortalized human cells (both non-transformed and cancer cells) identified members of the chaperome as some of the most abundant proteins in these cells (Finka and Goloubinoff 2013). HSP90s were the most abundant, averaging 2.8% alone and together with the HSP70s up to 5.5% the total protein mass. In light of the list of 147 chaperome members, these proteins together contributed 7.6% of the total number of polypeptides and 10.3% of the total protein mass in HeLa cells. The HSP60 and HSP110 chaperones accounted for another 3.3% of total protein mass, and 1.5% of total mass consisted mostly of regulatory cochaperones of the HSP90 and HSP70 machineries. More specifically, the HSP90AA1 and HSP90AB1 (HSP90α and HSP90β) isoforms and two HSP70s, the constitutive HSPA8 (heat shock cognate 70, HSC70) and the heat-inducible HSPA1A/B proteins represented the overwhelming majority of HSP90s and HSP70s in the cytosol. In addition, all known HSP90 co-chaperones were substoichiometric to HSP90. The co-chaperone to HSP90 ratio was 1:34 for AHA1 (an HSP90 ATPase activity activator) (Panaretou et al. 2002), 1:46 for CDC37 (the co-chaperone that links HSP90 to kinases) (Verba and Agard 2017), and 1:16 for HOP (HSP70-HSP90 organizing protein, also called STIP1, that links HSP90 to HSP70) (Carrigan et al. 2006). Similarly, the co-chaperone to HSP70 ratio was 1:5.5 for the various J-domain co-chaperones (that direct HSP70 to specific functions) (Kampinga and Craig 2010), 1:7 for HSP110s (which act as nucleotide exchange factors (NEFs) for HSP70 but also have independent chaperone functions and direct HSP70 for specific activities) (Shaner and Morano 2007), and 1:19 for the BAG proteins (which may also act as HSP70 NEFs or direct HSP70 for specific activities) (Bracher and Verghese 2015).

The organization of these chaperones and co-chaperones is in the form of cooperating protein networks (Brehme et al. 2019; Kumar et al. 2018; Rizzolo and Houry 2019; Voisine et al. 2010). Distinct and independent chaperome networks exist in eukaryotes, whereby a main chaperone, such as HSP90 or HSP70, functions with the aid of a number of co-chaperones, each with a dedicated set of functions (Albanese et al. 2006; Buchberger et al. 1996; Diezmann 2014; Garcia and Morano 2014; Hartl et al. 2011; Horwich 2014; Rospert and Chacinska 2006). In human cells, most studied, and understood, chaperome networks are those of the cytosolic HSP90 and HSP70 (Goloubinoff 2017; Mayer and Bukau 2005; Schopf et al. 2017). The past decade has seen a number of excellent papers report on the identity of chaperome network components, with studies in yeast leading the way (Albanese et al.

2006; Echeverria et al. 2011; Echtenkamp et al. 2011; Gong et al. 2009; Korcsmaros et al. 2007; McClellan et al. 2007; Rizzolo et al. 2017; Sun et al. 2015). Experimental advances have now expanded this knowledge to human disease (Hadizadeh Esfahani et al. 2018; Kishinevsky et al. 2018; Rodina et al. 2016; Taipale et al. 2014; Weidenauer et al. 2017). Important work was also published on cellular stress and how it may remodel chaperome networks (Brehme et al. 2014; Jacob et al. 2017; Kishinevsky et al. 2018; O'Meara et al. 2019; Palotai et al. 2008; Rodina et al. 2016; Truman et al. 2015). The goal of this chapter is not to review such large body of work, but rather to highlight studies into how chaperome networks influence cellular vulnerability in cancer. We focus on the HSP90 and HSP70 chaperome networks and discuss factors that portend sensitivity, or the lack of, to inhibition of chaperome network components.

6.2 Chaperome Networks

With the advent of datasets from large-scale genomic and proteomic analyses, several chaperome interactomes and network analyses were reported in yeast and other organisms (Echeverria et al. 2011; Echtenkamp et al. 2011; Gong et al. 2009; Korcsmaros et al. 2007). These studies identify chaperones as hubs, which are highly connected proteins in a protein-protein interaction network, but also as connectors of hubs, indicating an ability to integrate distinct cellular processes. These studies also suggest that interactions of the chaperones with network components are of low affinity and transient, perhaps reflecting a dynamic character and an ability to quickly rewire the network during stress to achieve system stability. By their central role in such protein networks, chaperones may also connect with a large number of network modules, a placement that indicates their ability to participate in a variety of distinct, and vital, cellular processes (Echeverria et al. 2011; Gong et al. 2009; Korcsmaros et al. 2007; Rizzolo and Houry 2019).

While networks display the versatility of the chaperome and its potential placement in the larger proteome network, networks do not necessarily provide information on the actual connectivity that the chaperome members establish among themselves or with the proteome, or how such connectivity may change in human cells. To address these factors, we will next interrogate the physical interaction, and integration, of distinct chaperome networks in human cells, in conditions of normal physiology and then in conditions of disease, such as in cancer.

Initial forays into the human chaperome networks have often been disappointing in their ability to generate the large interactomes expected for a hub protein such as HSP90 or HSP70 (Hartson and Matts 2012; Weidenauer et al. 2017). The dynamic nature of the chaperome-interactome and the poor suitability of available experimental tools, have been a major impediment. An advance came from the introduction of the LUMIER assays to quantitatively characterize interactions between chaperones, co-chaperones and putative interactors (referred to as 'clients') (Taipale et al. 2012, 2014). Originally developed by the Wrana lab (Barrios-Rodiles et al. 2017), LUMIER takes advantage of the sensitivity and linear range of luciferases. In a large-scale study conducted in HEK293T cells, several tagged chaperome members were introduced exogenously and then their potential interactors were investigated using LUMIER (Taipale et al. 2014). Among the analyzed chaperome components were cytosolic HSP90s and HSP70s, and over 50 co-factors and co-chaperones. The study found that while both HSP90 and HSP70 were hubs of protein networks, they functioned separately, each with its cochaperone subset and each as a hub of its own protein network. This finding was later confirmed by Rodina et al. who used chemoproteomics affinity-purification approaches to identify the chaperome and its interactome in a number of non-transformed cells and cancer cells, and then validated the finding through a variety of alternative methods (Rodina et al. 2016). Collectively, these studies indicate that in human cells, the HSP90 and the HSP70 chaperome networks perform specialized

functions through subsets of co-chaperones and moreover, behave like insular chaperome communities, with little physical and functional overlap. This insular behavior is however lost in a number of cancer cells, but not all, and we will reconvene on this topic further below in section "Stress limits redundancy".

6.3 Chaperome Network Redundancy

We next will discuss how insularity gives way to redundancy in chaperome networks (Fig. 6.1). The goal of redundancy is to prevent or recover from the failure of a specific component or pathway. We therefore often hear about network redundancy and its implementation in every aspect of life. For example, high traffic web servers and other critical systems may have multiple power supplies that take over in case the primary one fails. Computer networks often implement redundancy, and from local area networks to Internet backbone connections, it is common to have redundant data paths. Power grids protect against failures by building redundant paths – if a line is damaged by wildfires or falling trees another can take over. The role of a redundant pathway or device is therefore to assure that, if one component fails, the connection between other systems will not be broken. Nature also introduces redundancy into cellular networks to improve reliability and enable cellular survival in the advent of continuous fluctuations in the extra- and intra-cellular environment (Navlakha et al. 2014).

Redundancy in the chaperome networks is evidenced in a number of large-scale investigations where individual chaperomes were either genetically deleted or pharmacologically inhibited. For example, a large-scale investigation of proteome changes following deletion of *SSA1* and *SSB1* (two HSP70 paralogs) was performed in yeast that were grown under optimal conditions (Jamuczak et al. 2015). Whereas Ssa1 is primarily involved in cellular house-keeping

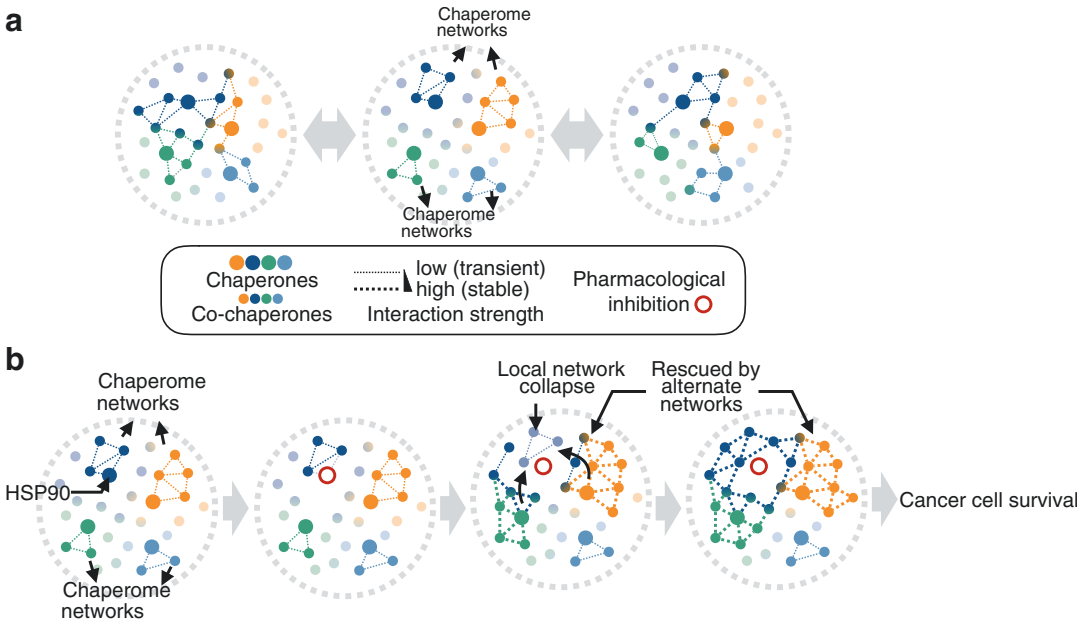

Fig. 6.1 Cancer cells with a global chaperome network composed of insular, partly overlapping, chaperome networks. (**a**) Fluctuations in the cellular environment are rapidly dispersed, and cellular function stabilized, by network rearrangement and workload transfer among networks. (**b**) The temporary impairment of a sub-network by drugs can be rescued by alternate subnetworks coming into play to take over the workload of the impaired chaperome. Cellular survival is maintained and cells recover after drug removal

functions, Ssb1 plays a dedicated role as a member of the Ribosome-Associated Complex. In addition to being highly abundant (both proteins are among the top 5% of yeast proteins by mass) (Jamuczak et al. 2015), Ssa1 and Ssb1 contain the most connections among all hub proteins, with 3269 and 2489 client-protein links, respectively, as well as interactions observed with over 40 other chaperones (Jamuczak et al. 2015). Surprisingly, no substantial changes in individual protein concentrations were associated with loss of *SSA1 and SSB1,* prompting the authors to suggest that the continuous function of the chaperome network following their loss is maintained by other chaperones taking on the workload, a process more cost effective than increasing the concentrations of other chaperones. This "functional takeover" could be done either by another HSP70 member (Ssa1 paralogs include Ssa2, Ssa3, and Ssa4, and Ssb2 is an Ssb1 paralog), or possibly by other chaperone machinery, such as the HSP90 network.

Such functional takeover was also evidenced in human cells. In a large-scale mass spectrometry-based method, the interactome of an HSP90 kinase, CDK4, was analyzed before and after inhibition with the HSP90 inhibitor NVP-AUY922 (Lambert et al. 2013). While in basal conditions CDK4 was identified in complex with HSP90α/β, CDC37 and two immonophilins (FKBP4 and 5), upon inhibition with NVP-AUY922 the kinase became bound to HSC70, HSP70 (HSPA1A), HOP and HIP. While the functional meaning of such transfer was not investigated, one may speculate that upon HSP90 inhibition, CDK4 may be scaffolded by the HSC70/HIP complex to slow its clearance. This has been observed for tau, where binding by HSC70 or HSP70 may slow or accelerate tau clearance, respectively (Jinwal et al. 2013).

A similar functional takeover was also observed when the HSP90 interactome was analyzed in HEK293T cells in the presence of ATP, ADP and geldanamycin (GM) (Gano and Simon 2010). As expected for a cell with insular chaperome networks, little connectivity was noted for HSP90 with the HSP70 network, as indicated by little to no isolated HSC70 (HSPA8), BAG proteins, HIP (ST13) and others on the HSP90 bait. Intriguingly, upon GM treatment, HSP90 association increased with these HSP70 chaperome members, and moreover was enhanced with other chaperome members such as CDC37, FKBP4, TTC9C, TTC4, DNAJC7, PIHD1, CD37L, RPAP3, and others.

The transfer of function from one chaperome network to another, and the rewiring of chaperome network paths upon the incapacitation of individual network nodes, are both evidence of chaperome network redundancy. This phenomenon was recently linked to the resistance of cancer cells to inhibition of important chaperone network nodes and components (Joshi et al. 2018; Rodina et al. 2016). In such cells with insular HSP90 and HSP70 networks, inhibition of either HSP90, AHA1, HOP or HSP110, pharmacologically or through siRNA knockdown, led to transient growth suppression but little cell death.

6.4 Stress Limits Redundancy

Thus, inbuilt redundancy in chaperome networks and the ensuing ability of chaperome networks to share the workload when a key chaperome component is inhibited, appears, at least in part, to foil single agent chaperome therapies in cancer. In practice, networks cannot be infinitely redundant and at a point, even fail-safe paths will be utilized by the ever increasing and persistent stresses (Fig. 6.2). This is a point of vulnerability – here, the entire network is occupied, no fail-safe paths exist, and the system will collapse under additional insult, such as an inhibitor of a key network component (Joshi et al. 2018).

There are two concepts, in analogy to power grids, to understand and discuss here in the context of chaperome networks. Stress may overburden a given chaperome network, and the capacity of another network will be permanently co-opted, as opposed to only deployed under an acute stress, to maintain cellular integrity. This is a cellular system where chaperome networks are no longer insular. Workload overspill creates a state of permanent chaperome network interconnection, or hyperconnectivity. In analogy to the

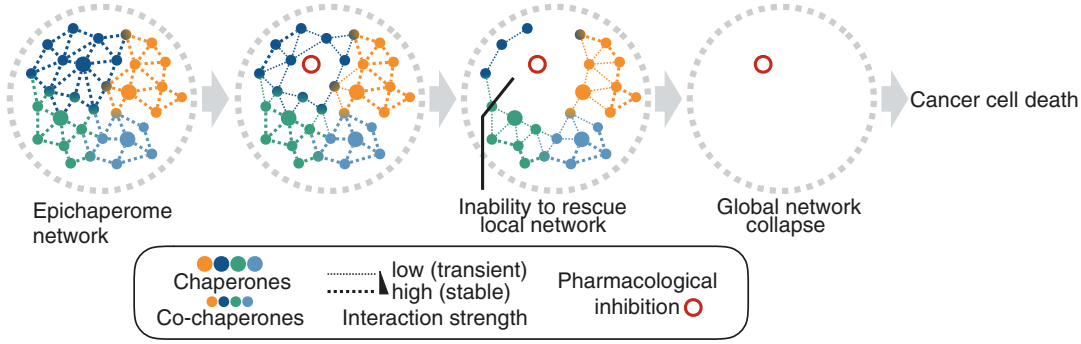

Fig. 6.2 Cancer cells with a highly interconnected global chaperome network. Certain cancer cells characterized by large proteome imbalances (such as induced by MYC hyperactivation) rewire individual chaperome networks into a hyperconnected cellular network, the epichaperome. Inhibition of key nodes in the epichaperome network propagates to the entire network and results in overall network collapse. Cells cannot survive epichaperome collapse and cell death ensues

power grid, such baseline overuse of fail-safe paths reduces the ability of the network to defend when further insult (*i.e*. chaperome inhibitor) is applied (Joshi et al. 2018; Rodina et al. 2016).

What stresses then may overburden the chaperome networks? Introduction into NIH3T3 cells of a *bona fide* HSP90 client, such as v-SRC or mutant MET kinase, is insufficient to induce chaperome network hyperconnectivity (Joshi et al. 2018; Rodina et al. 2016). MYC hyperactivation however induces a hyperconnectivity state, and rewires the HSP90 and HSP70 chaperome networks into a large functionally- and physically-integrated network (Joshi et al. 2018; Kourtis et al. 2018; Rodina et al. 2016). Both the HSP90α and HSP90β paralogues, but mainly HSC70 and not HSP70 (the inducible HSP70 paralogue also known as HSP72 or HSP70-1) participate in the creation of the hyperconnected HSP90-HSP70 chaperome network. MYC exogenous introduction and knock-down is sufficient to connect and disconnect, respectively, the chaperome networks (Joshi et al. 2018; Rodina et al. 2016). NOTCH1, which acts as an upstream activator of MYC in T-cell acute lymphoblastic leukemia (T-ALL), also induces chaperome network hyperconnectivity (Kourtis et al. 2018). In aggressive acute myeloid leukemias we found a direct and quantitative link between hyperactivation of signaling pathways and chaperome network connectivity (Rodina et al. 2016; Zong et al. 2015).

How is chaperome network hyperconnectivity achieved? Evidence indicates that changes in the interaction strength and partners of the major chaperones HSP90 and HSC70, which is not necessarily accompanied by changes in their expression levels, can result in the formation of stable macromolecular structures (Rodina et al. 2016). These structures act as molecular scaffolding platforms that bring together the components of the 'chaperome' and of the 'proteome' into cell-wide hyperconnected networks. As such their function is not in folding *per se*, but, rather, in increasing cellular adaptation to the stress of cancer by augmenting the fitness of oncogenic protein networks and pathways (Joshi et al. 2018). We coined the term "epichaperome" to describe such stress-specific chaperome pools that are distinct in structure, dynamics, and function from the physiologic chaperome units (Joshi et al. 2018; Rodina et al. 2016; Tai et al. 2016).

In cancer cells, disruption of the epichaperome networks by siRNA knock-down or pharmacologic inhibition of one of the network's nodes, *e.g*. HSP90, HSP110, HOP and AHA1, resulted in cancer cell lethality (Rodina et al. 2016). Turning MYC on and off, rendered cancer cells sensitive or resistant, respectively, to node inhibition (Rodina et al. 2016). In T-ALL, where MYC activity is regulated by NOTCH1, inhibition

of NOTCH1 by a γ-secretase inhibitor mimicked the effect of MYC knock-down, in that it reverted the chaperome networks to insular, and in turn rendered cells insensitive to chaperome network node inhibition (Kourtis et al. 2018).

Yeast under heat stress also behaves like a cellular system where chaperome interconnectivity imparts vulnerability. Yeast that tolerate the lack of Sti1 (the yeast HOP) and Sse1 (the yeast HSP110) at 30 °C, could not grow at 37 °C. Deletion of the Sse1 however, when also associated with loss of HSP82 (the yeast HSP90 homolog) was toxic even at 30 °C (Liu et al. 1999). Sti1 (the yeast HOP) or Sse1 (the yeast HSP110) mutant strains exhibited markedly increased sensitivity to inhibition of HSP90 by geldanamycin or macbecin in conditions in which the wild type strain remained unaffected by these drugs (Liu et al. 1999).

These results, combined, can be viewed as further evidence of chaperome network connectivity or redundancy. For example, in normal growth conditions (yeast) or normal cellular function (mammalian cell) HSP90 and its co-chaperones exist as a community that only partially interacts, or communicates with, the HSP70 chaperome network. This partial interconnectivity may be manifested via HOP or other chaperome members. HSP90 impairment alone has little effect on cellular viability because its function may be supplanted by the HSP70 network (and possibly others). The reverse may be also true when HSP70 is incapacitated. In HOP defective yeast, functional transfer is impaired, so the strains are more sensitive to both heat and HSP90 inhibition. In HSP110 deficient yeast, while transfer via HOP is possible, activation of the holdase activity of the HSP70 machinery is impaired without the contribution of HSP110, which also exhibits holdase activity.

To conclude this section, interchaperome network communication is used, and necessary, for cancer cell function under stress but also renders these cells more vulnerable to additional insults when chaperome components are impaired. The essentiality of a chaperome member, and in turn the vulnerability of a cellular system to its loss, is therefore measured by the chaperome's connectivity. The chaperome becomes essential when its network connections increase through engagement in protein complexes with chaperome members of other chaperome machineries. The increased interactions allow the previously nonessential chaperome to become a member of global (as opposed to insular) protein pathways.

6.5 Less Redundancy, More Vulnerability

While the chaperones HSP90 and HSC70 mediate chaperome network interconnection, only a fraction of the total chaperome in the cancer cell, and a small proportion of the chaperome in the human body participates in this process (Kishinevsky et al. 2018; Pillarsetty et al. 2019; Rodina et al. 2016). In cancer cells, the more the chaperome participated in the formation of the interconnected chaperome networks, that is the epichaperome, the more sensitive the tumor cell became to pharmacologic or genetic chaperome modulators (Joshi et al. 2018; Kourtis et al. 2018; Rodina et al. 2016). By analyzing 95 cancer cell lines (representing pancreatic, gastric, lung, and breast cancers, as well as lymphomas and leukemias), 40 primary AMLs and 23 primary breast tumors *ex vivo* and 51 solid tumors and lymphomas, *in vivo*, in patients, it was found that 50–60% expressed variable epichaperome levels and ~10–15% were high expressors, as defined by the amount of HSP90 residing in epichaperome networks. Epichaperome abundance was independent of tissue of origin, tumor subtype or genetic background (Pillarsetty et al. 2019; Rodina et al. 2016).

In the context of chaperome network connectivity discussed above, the high-epichaperome expressing tumors characterize a state of maximal chaperome network occupancy, where all paths of the interconnected chaperome networks are deployed. These findings have major implications for cancer treatment. They advise a change in our mentality of how to implement chaperome inhibitors in cancer, and propose a fresh look at the chaperome that is based on a

novel mechanistic understanding of chaperome network interconnectivity. In this context, HSP90 inhibition is lethal only when HSP90 is hyperconnected with the HSP70 machinery and other chaperomes, to form epichaperomes. Single agent chaperome therapies are therefore more likely to succeed in the 10–15% of high-epichaperome expressing tumors, *i.e.* tumors characterized by fully-occupied hyperconnected chaperome networks (Pillarsetty et al. 2019). Yet understanding that chaperome connectivity provides vulnerability, and that stress regulates such connectivity, provides insights into combinatorial strategies for the 55–60% of tumors that lie in between (*i.e*. depend on the epichaperome, but the interconnected network is not fully occupied, in other words, not all paths are deployed). One may imagine that in such tumors, pharmacologic means can be used to *in situ* increase the cellular content of the chaperome that switches into the epichaperome, creating a state of maximal chaperome network occupancy, and in turn of maximal vulnerability.

6.6 Chaperome Hyperconnectivity as a Biomarker

The finding that the more the chaperome is rewired into the epichaperome, the higher the sensitivity of a tumor to PU-H71 (or other means of epichaperome inhibition) places the epichaperome as a potential biomarker for clinical trial enrichment (Joshi et al. 2018). This biomarker is identifiable (*i.e*. clinically available assay can detect it) and actionable (*i.e*. clinically available drugs can target it) (Joshi et al. 2018; Pillarsetty et al. 2019) (Fig. 6.3).

PU-H71 is an inhibitor of HSP90 specifically when HSP90 is part of the stable chaperome complexes of epichaperome networks formed under stress (Rodina et al. 2016). It dissociates from epichaperomes much more slowly (*i.e*. over days) than it does from other HSP90 pools (*i.e.* minutes to hours); this difference in the k_{off} (*i.e*. dissociation constant) provides it with epichaperome selectivity (Rodina et al. 2016; Taldone et al. 2019; Wang et al. 2019). Its selectivity for the epichaperome over HSP90 has been shown in cell homogenates, in live cells, in mice and in humans, and through a number of alternative methods. While initially discovered as an HSP90 inhibitor, extensive studies by us and others have shown that PU-H71 prefers "a tumor enriched HSP90" or a "stress HSP90" (Bhagwat et al. 2014; Darby and Workman 2011; Goldstein et al. 2015; Kucine et al. 2015; Moulick et al. 2011; Nayar et al. 2013; Ojala 2013; Shrestha et al. 2016; Taldone et al. 2013, 2014; Zong et al. 2015). Follow-up studies have revealed that this HSP90 species is the cellular fraction residing in the epichaperome (Kishinevsky et al. 2018; Rodina et al. 2016). Owing to these features, PU-H71 itself is being tested in the clinic to treat epichaperome addicted tumors or to detect epichaperome-expressing tumors (ClinicalTrials.gov: NCT01393509, NCT03166085) (Gerecitano et al. 2013; Goldstein et al. 2015; Jhaveri et al. 2019; Pillarsetty et al. 2019; Roboz et al. 2018; Speranza et al. 2018).

Three assays were developed and translated to clinic to detect the epichaperome in patients – PU-PET for solid tumors by positron emission tomography (PET) imaging (ClinicalTrials.gov: NCT01269593, (Rodina et al. 2016)), PU-FITC for liquid tumors to detect the epichaperome by flow cytometry (Rodina et al. 2016; Zong et al. 2015) and IEF for biopsies to detect the epichaperome by native IEF chromatography (Rodina et al. 2016). The first two assays make use of relevantly labeled versions of PU-H71 to detect the epichaperome by flow cytometry (PU-FITC assay, (Roboz et al. 2018; Rodina et al. 2016; Zong et al. 2015)) or to detect epichaperomes in solid tumors by PET imaging (PU-PET assay, ClinicalTrials.gov: NCT01269593, (Jhaveri et al. 2019; Rodina et al. 2016)).

Both preclinical and clinical data support a significant correlation between epichaperome abundance and vulnerability of tumors to its inhibition (Jhaveri et al. 2019; Pillarsetty et al. 2019; Roboz et al. 2018; Rodina et al. 2016). Combined with findings that epichaperome expression is independent of genetics and tumor type (Rodina et al. 2016), these suggest that basket trials where

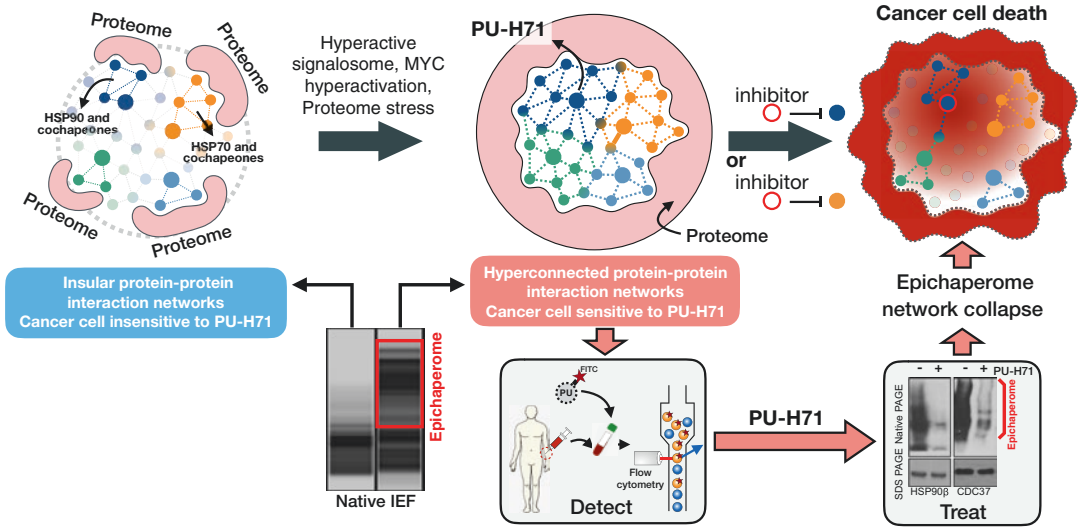

Fig. 6.3 Paradigm for a chaperome network-driven approach to cancer therapy

epichaperome abundance is used as criteria for patient selection are more suitable for chaperome therapies such as PU-H71 than the classical disease focused studies (*i.e.* breast cancer or pancreatic cancer, for example).

6.7 Conclusions and Future Outlook

We have discussed how in human tumors, evolution under the stress associated with malignant transformation has led to divergent mechanisms by which chaperome networks regulate proteostasis. In one state, chaperome networks work in isolation and with little overlap, and is a cellular state that resembles normal, non-transformed, cells (Fig. 6.1). This is not a state amenable for single-agent chaperome therapy due to redundancy in chaperome networks. In the other state, cellular survival under stress requires and relies on chaperome network interconnectivity. Certain stresses, such as MYC hyperactivation, drive maximal chaperome network interconnectivity (Fig. 6.2). This is a state where no redundant pathways may be deployed; it is a state of vulnerability, amenable for chaperome therapy.

This chaperome network approach to therapy challenges the current view of how inhibitors, such as those that target HSP90 or HSP70, are developed in cancer (Fig. 6.3). First, it emphasizes that properties of chaperome networks, not genetics or individual client proteins, should drive chaperome therapy implementation. It proposes a blueprint for the translation of inhibitors of hub chaperome members to clinic based on the context-dependent vulnerability of tumors to chaperome networks. Second, it highlights the need, and the ability to, develop inhibitors that differentiate the chaperome variants residing in epichaperomes from those involved in normal homeostasis. Third, it offers the potential for precision medicine, where the aberrant epichaperomes act as actionable biomarkers for patient selection. Altogether, it proposes that chaperome network hyperconnectivity is a target of intervention in cancer, a target agnostic to genetics and client proteins. In light of these findings, previous disappointments with HSP90 inhibitors in cancer may not be surprising.

These chaperome network-driven mechanisms also opens the door to strategies that artificially increase chaperome networks' connectivity, and in turn limit redundancy, to induce tumor vulnerability. To identify such strategies, many outstanding questions remain to be answered. How do epichaperomes form? Identification of factors that are sufficient and required for epichaperome formation will be important here because activating the epichaperome can induce

a synthetic vulnerability to epichaperome inhibitors. How may we influence the formation of the epichaperome *in situ*? Identification of required factors and pathways linking MYC, for example, and epichaperome formation may provide a clue to this question. Finding genes or protein pathways whose inactivation or activation are sufficient to drive epichaperome formation is also key. These approaches may lead to strategies to induce epichaperomes and epichaperome inhibitor sensitivity in cancer cells. Large-scale efforts are most suitable to address and tackle such complexity, and thus application of unbiased genomic and proteomic approaches are needed to dissect the emerging biology of epichaperome activity in cancer.

What therapies positively (or negatively) influence epichaperome formation? Identification of drugs that may augment epichaperome formation *in situ* is important because it may result in combination approaches with immediate clinical translation in cancer. Knowing which drugs may inhibit or decrease the epichaperome is also important as these may negatively affect epichaperome therapies (*i.e.* many patients while on an epichaperome inhibitor, may also be taking drugs for other ailments, and these may interfere with their cancer treatment).

What is the composition and the design of a combination therapy that builds on the epichaperome for maximal therapeutic benefit? Therapies built around epichaperome inhibitors will need to investigate the effect of combination therapies on epichaperome networks. In such instances, the sequence of therapy administration will likely play an important role in optimizing potency and efficacy, as therapies may either increase or decrease chaperome connectivity, and in turn the effectiveness of epichaperome inhibitors. In addition, treatment is the balance between target engagement, therapeutic index, modulation of the tumor microenvironment and an enhancement of the immune response. Therefore, the efficacy and safety of sequential treatment strategies in cancer will need to investigate how they influence and are influenced by the tumor microenvironment and the immune system.

This mechanism of increased chaperome network connectivity under pathologic stress, and its execution through increased participation of chaperome members in protein complexes of enhanced stability, is not restricted to cancer and we recently reported it the context of iPSC-derived neuronal models of Parkinson's disease (Kishinevsky et al. 2018). In neurons carrying a PARKIN mutant, the switch from chaperome into interconnected chaperome networks mediated aberrant activities in several protein pathways, with a detrimental outcome on neuronal function (Kishinevsky et al. 2018). Importantly, pharmacological inhibition of the stress-rewired chaperome networks by PU-H71 reversed abnormal proteome-wide activity to normal levels and rescued the viability of the neurons.

In conclusion, chaperome network essentiality (*i.e.* chaperome rewiring into epichaperomes) expands the existing concepts for therapeutic strategies to provide a framework for the discovery of cancer-specific vulnerabilities. The context-dependent nature of chaperome essentiality can be exploited to develop more effective and more specific chaperome targeted therapies and provides avenues for patient-tailored anticancer therapies. Rewiring of chaperome networks by increasing connectivity may be a general mechanism of stress-induced pathologic proteome alterations that is both identifiable and actionable.

Acknowledgements G.C. is supported by the US National Institutes of Health (NIH) (R01 CA172546, R56 AG061869, R01 CA155226, P01 CA186866, P30 CA08748 and P50 CA192937), the Steven A. Greenberg charitable trust, the Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research and the Experimental Therapeutics Center of the Memorial Sloan Kettering Cancer Center; T.W. is supported by the Lymphoma Research Foundation; M.L.G. is supported by the NIH (R01 CA172546, R01 CA102031), the Irma T. Hirschl/Monique Weill-Caulier Trust and Unravel Pediatric Cancer Foundation.

Author Contributions P.Y., R.I.P. and M.L.G. researched data for the article and contributed to the writing of the article and to the review of the manuscript. T.W. designed the figures and their content. G.C. designed the content of the manuscript, researched data for the article and wrote, edited and reviewed the manuscript.

Declaration of Interests Memorial Sloan Kettering Cancer Center holds the intellectual rights to the epichaperome portfolio. Samus Therapeutics, of which G.C. has partial ownership and is a member of its board of directors, has licensed the portfolio. G.C., P.Y. and M.L.G. are inventors on the licensed intellectual property. All other authors declare no competing interests.

References

Albanese V, Yam AY, Baughman J, Parnot C, Frydman J (2006) Systems analyses reveal two chaperone networks with distinct functions in eukaryotic cells. Cell 124:75–88

Barrios-Rodiles M, Ellis JD, Blencowe BJ, Wrana JL (2017) LUMIER: a discovery tool for mammalian protein interaction networks. Methods Mol Biol 1550:137–148

Bhagwat N, Koppikar P, Keller M, Marubayashi S, Shank K, Rampal R, Qi J, Kleppe M, Patel HJ, Shah SK et al (2014) Improved targeting of JAK2 leads to increased therapeutic efficacy in myeloproliferative neoplasms. Blood 123:2075–2083

Bracher A, Verghese J (2015) The nucleotide exchange factors of Hsp70 molecular chaperones. Front Mol Biosci 2:10

Brehme M, Voisine C (2016) Model systems of protein-misfolding diseases reveal chaperone modifiers of proteotoxicity. Dis Model Mech 9:823–838

Brehme M, Voisine C, Rolland T, Wachi S, Soper JH, Zhu Y, Orton K, Villella A, Garza D, Vidal M et al (2014) A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep 9:1135–1150

Brehme M, Sverchkova A, Voisine C (2019) Proteostasis network deregulation signatures as biomarkers for pharmacological disease intervention. Curr Opin Syst Biol 15:74–81

Buchberger A, Schroder H, Hesterkamp T, Schonfeld HJ, Bukau B (1996) Substrate shuttling between the DnaK and GroEL systems indicates a chaperone network promoting protein folding. J Mol Biol 261:328–333

Carrigan PE, Sikkink LA, Smith DF, Ramirez-Alvarado M (2006) Domain:domain interactions within Hop, the Hsp70/Hsp90 organizing protein, are required for protein stability and structure. Protein Sci 15:522–532

Czarnecka AM, Campanella C, Zummo G, Cappello F (2006) Mitochondrial chaperones in cancer: from molecular biology to clinical diagnostics. Cancer Biol Ther 5:714–720

Darby JF, Workman P (2011) Chemical biology: many faces of a cancer-supporting protein. Nature 478:334–335

Diezmann S (2014) The Candida albicans Hsp90 chaperone network is environmentally flexible and evolutionarily divergent. In: Houry WA (ed) The molecular chaperones interaction networks in protein folding and degradation. Springer, New York, pp 185–206

Echeverria PC, Bernthaler A, Dupuis P, Mayer B, Picard D (2011) An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine. PLoS One 6:e26044

Echtenkamp FJ, Zelin E, Oxelmark E, Woo JI, Andrews BJ, Garabedian M, Freeman BC (2011) Global functional map of the p23 molecular chaperone reveals an extensive cellular network. Mol Cell 43:229–241

Finka A, Goloubinoff P (2013) Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. Cell Stress Chaperones 18:591–605

Finka A, Mattoo RU, Goloubinoff P (2011) Meta-analysis of heat- and chemically upregulated chaperone genes in plant and human cells. Cell Stress Chaperones 16:15–31

Gano JJ, Simon JA (2010) A proteomic investigation of ligand-dependent HSP90 complexes reveals CHORDC1 as a novel ADP-dependent HSP90-interacting protein. Mol Cell Proteomics 9:255–270

Garcia VM, Morano KA (2014) The chaperone networks: a heat shock protein (Hsp)70 perspective. In: Houry WA (ed) The molecular chaperones interaction networks in protein folding and degradation. Springer New York, New York, pp 83–108

Gerecitano JF, Modi S, Gajria D, Taldone T, Alpaugh M, DaGama EG, Uddin M, Chiosis G, Lewis JS, Larson SM et al (2013) Using 124I-PU-H71 PET imaging to predict intratumoral concentration in patients on a phase I trial of PU-H71. J Clin Oncol 31:11076

Goldstein RL, Yang SN, Taldone T, Chang B, Gerecitano J, Elenitoba-Johnson K, Shaknovich R, Tam W, Leonard JP, Chiosis G et al (2015) Pharmacoproteomics identifies combinatorial therapy targets for diffuse large B cell lymphoma. J Clin Invest 125:4559–4571

Goloubinoff P (2017) Editorial: the HSP70 molecular chaperone machines. Front Mol Biosci 4:1

Gong Y, Kakihara Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA (2009) An atlas of chaperone-protein interactions in Saccharomyces cerevisiae: implications to protein folding pathways in the cell. Mol Syst Biol 5:275

Hadizadeh Esfahani A, Sverchkova A, Saez-Rodriguez J, Schuppert AA, Brehme M (2018) A systematic atlas of chaperome deregulation topologies across the human cancer landscape. PLoS Comput Biol 14:e1005890

Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475:324–332

Hartson SD, Matts RL (2012) Approaches for defining the Hsp90-dependent proteome. Biochim Biophys Acta 1823:656–667

Horwich AL (2014) Molecular chaperones in cellular protein folding: the birth of a field. Cell 157:285–288

Jacob P, Hirt H, Bendahmane A (2017) The heat-shock protein/chaperone network and multiple stress resistance. Plant Biotechnol J 15:405–414

Jamuczak AE, Eyers CE, Schwartz JM, Grant CM, Hubbard SJ (2015) Quantitative proteomics and network analysis of SSA1 and SSB1 deletion mutants reveals robustness of chaperone HSP70 network in Saccharomyces cerevisiae. Proteomics 15:3126–3139

Jhaveri K, Dunphy M, Wang R, Comen E, Fornier M, Moynahan ME, Bromberg J, Ma W, Patil S, Taldone T et al (2019) Tumor epichaperome expression using 124I PU-H71 PET (PU-PET) as a biomarker of response for PU-H71 plus nab-paclitaxel in HER2 negative (HER2-) metastatic breast cancer (MBC). Cancer Res 79:P6-20-03

Jinwal UK, Akoury E, Abisambra JF, O'Leary JC 3rd, Thompson AD, Blair LJ, Jin Y, Bacon J, Nordhues BA, Cockman M et al (2013) Imbalance of Hsp70 family variants fosters tau accumulation. FASEB J 27:1450–1459

Joshi S, Wang T, Araujo TLS, Sharma S, Brodsky JL, Chiosis G (2018) Adapting to stress – chaperome networks in cancer. Nat Rev Cancer 18:562–575

Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592

Kishinevsky S, Wang T, Rodina A, Chung SY, Xu C, Philip J, Taldone T, Joshi S, Alpaugh ML, Bolaender A et al (2018) HSP90-incorporating chaperome networks as biosensor for disease-related pathways in patient-specific midbrain dopamine neurons. Nat Commun 9:4345

Korcsmaros T, Kovacs IA, Szalay MS, Csermely P (2007) Molecular chaperones: the modular evolution of cellular networks. J Biosci 32:441–446

Kourtis N, Lazaris C, Hockemeyer K, Balandran JC, Jimenez AR, Mullenders J, Gong Y, Trimarchi T, Bhatt K, Hu H et al (2018) Oncogenic hijacking of the stress response machinery in T cell acute lymphoblastic leukemia. Nat Med 24:1157–1166

Kucine N, Marubayashi S, Bhagwat N, Papalexi E, Koppikar P, Sanchez Martin M, Dong L, Tallman MS, Paietta E, Wang K et al (2015) Tumor-specific HSP90 inhibition as a therapeutic approach in JAK-mutant acute lymphoblastic leukemias. Blood 126:2479–2483

Kumar A, Rizzolo K, Zilles S, Babu M, Houry WA (2018) Computational analysis of the chaperone interaction networks. Methods Mol Biol 1709:275–291

Lambert JP, Ivosev G, Couzens AL, Larsen B, Taipale M, Lin ZY, Zhong Q, Lindquist S, Vidal M, Aebersold R et al (2013) Mapping differential interactomes by affinity purification coupled with data-independent mass spectrometry acquisition. Nat Methods 10:1239–1245

Lee AS (2014) Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer 14:263–276

Liu XD, Morano KA, Thiele DJ (1999) The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. J Biol Chem 274:26654–26660

Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684

McClellan AJ, Xia Y, Deutschbauer AM, Davis RW, Gerstein M, Frydman J (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. Cell 131:121–135

Moulick K, Ahn JH, Zong H, Rodina A, Cerchietti L, Gomes DaGama EM, Caldas-Lopes E, Beebe K, Perna F, Hatzi K et al (2011) Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. Nat Chem Biol 7:818–826

Navlakha S, He X, Faloutsos C, Bar-Joseph Z (2014) Topological properties of robust biological and computational networks. J R Soc Interface 11:20140283

Nayar U, Lu P, Goldstein RL, Vider J, Ballon G, Rodina A, Taldone T, Erdjument-Bromage H, Chomet M, Blasberg R et al (2013) Targeting the Hsp90-associated viral oncoproteome in gammaherpesvirus-associated malignancies. Blood 122:2837–2847

O'Meara TR, O'Meara MJ, Polvi EJ, Pourhaghighi MR, Liston SD, Lin ZY, Veri AO, Emili A, Gingras AC, Cowen LE (2019) Global proteomic analyses define an environmentally contingent Hsp90 interactome and reveal chaperone-dependent regulation of stress granule proteins and the R2TP complex in a fungal pathogen. PLoS Biol 17:e3000358

Ojala PM (2013) Naughty chaperone as a target for viral cancer. Blood 122:2767–2768

Palotai R, Szalay MS, Csermely P (2008) Chaperones as integrators of cellular networks: changes of cellular integrity in stress and diseases. IUBMB Life 60:10–18

Panaretou B, Siligardi G, Meyer P, Maloney A, Sullivan JK, Singh S, Millson SH, Clarke PA, Naaby-Hansen S, Stein R et al (2002) Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. Mol Cell 10:1307–1318

Pillarsetty N, Jhaveri K, Taldone T, Caldas-Lopes E, Punzalan B, Joshi S, Bolaender A, Uddin MM, Rodina A, Yan P et al (2019) Paradigms for precision medicine in epichaperome cancer therapy. Cancer Cell 36(5):559–573.e7

Richter K, Haslbeck M, Buchner J (2010) The heat shock response: life on the verge of death. Mol Cell 40:253–266

Ritossa F (1962) A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18:571–573

Ritossa FM (1964) Experimental activation of specific loci in polytene chromosomes of Drosophila. Exp Cell Res 35:601–607

Rizzolo K, Houry WA (2019) Multiple functionalities of molecular chaperones revealed through systematic mapping of their interaction networks. J Biol Chem 294:2142–2150

Rizzolo K, Huen J, Kumar A, Phanse S, Vlasblom J, Kakihara Y, Zeineddine HA, Minic Z, Snider J, Wang W et al (2017) Features of the chaperone cellular network revealed through systematic interaction mapping. Cell Rep 20:2735–2748

Roboz GJ, Sugita M, Mosquera JM, Wilkes DC, Nataraj S, Jimenez-Flores RA, Bareja R, Yan L, Eng KW, Croyle JA et al (2018) Targeting the epichaperome as an effective precision medicine approach in a novel PML-SYK fusion acute myeloid leukemia. Blood 132:1435

Rodina A, Wang T, Yan P, Gomes ED, Dunphy MP, Pillarsetty N, Koren J, Gerecitano JF, Taldone T, Zong H et al (2016) The epichaperome is an integrated chaperome network that facilitates tumour survival. Nature 538:397–401

Rospert S, Chacinska A (2006) Distinct yet linked: chaperone networks in the eukaryotic cytosol. Genome Biol 7:208

Schopf FH, Biebl MM, Buchner J (2017) The HSP90 chaperone machinery. Nat Rev Mol Cell Biol 18:345–360

Shaner L, Morano KA (2007) All in the family: atypical Hsp70 chaperones are conserved modulators of Hsp70 activity. Cell Stress Chaperones 12:1–8

Shrestha L, Patel HJ, Chiosis G (2016) Chemical tools to investigate mechanisms associated with HSP90 and HSP70 in disease. Cell Chem Biol 23:158–172

Speranza G, Anderson L, Chen AP, Do K, Eugeni M, Weil M, Rubinstein L, Majerova E, Collins J, Horneffer Y et al (2018) First-in-human study of the epichaperome inhibitor PU-H71: clinical results and metabolic profile. Invest New Drugs 36:230–239

Sun L, Hartson SD, Matts RL (2015) Identification of proteins associated with Aha1 in HeLa cells by quantitative proteomics. Biochim Biophys Acta 1854:365–380

Tai W, Guzman ML, Chiosis G (2016) The epichaperome: the power of many as the power of one. Oncoscience 3:266–267

Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 150:987–1001

Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC, Lindquist S (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. Cell 158:434–448

Taldone T, Rodina A, DaGama Gomes EM, Riolo M, Patel HJ, Alonso-Sabadell R, Zatorska D, Patel MR, Kishinevsky S, Chiosis G (2013) Synthesis and evaluation of cell-permeable biotinylated PU-H71 derivatives as tumor Hsp90 probes. Beilstein J Org Chem 9:544–556

Taldone T, Ochiana SO, Patel PD, Chiosis G (2014) Selective targeting of the stress chaperome as a therapeutic strategy. Trends Pharmacol Sci 35:592–603

Taldone T, Wang T, Rodina A, Pillarsetty NVK, Digwal CS, Sharma S, Yan P, Joshi S, Pagare PP, Bolaender A et al (2019) A chemical biology approach to the chaperome in cancer-HSP90 and beyond. Cold Spring Harb Perspect Biol

Truman AW, Kristjansdottir K, Wolfgeher D, Ricco N, Mayampurath A, Volchenboum SL, Clotet J, Kron SJ (2015) Quantitative proteomics of the yeast Hsp70/Hsp90 interactomes during DNA damage reveal chaperone-dependent regulation of ribonucleotide reductase. J Proteomics 112:285–300

Verba KA, Agard DA (2017) How Hsp90 and Cdc37 lubricate kinase molecular switches. Trends Biochem Sci 42:799–811

Voisine C, Pedersen JS, Morimoto RI (2010) Chaperone networks: tipping the balance in protein folding diseases. Neurobiol Dis 40:12–20

Voos W, Rottgers K (2002) Molecular chaperones as essential mediators of mitochondrial biogenesis. Biochim Biophys Acta 1592:51–62

Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, Gurkan C, Kellner W, Matteson J, Plutner H et al (2006) Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 127:803–815

Wang T, Rodina A, Dunphy MP, Corben A, Modi S, Guzman ML, Gewirth DT, Chiosis G (2019) Chaperome heterogeneity and its implications for cancer study and treatment. J Biol Chem 294:2162–2179

Weidenauer L, Wang T, Joshi S, Chiosis G, Quadroni MR (2017) Proteomic interrogation of HSP90 and insights for medical research. Expert Rev Proteomics 14:1105–1117

Zong H, Gozman A, Caldas-Lopes E, Taldone T, Sturgill E, Brennan S, Ochiana SO, Gomes-DaGama EM, Sen S, Rodina A et al (2015) A hyperactive signalosome in acute myeloid leukemia drives addiction to a tumor-specific Hsp90 species. Cell Rep 13:2159–2173

7 The Role of HSF1 and the Chaperone Network in the Tumor Microenvironment

Nil Grunberg, Oshrat Levi-Galibov, and Ruth Scherz-Shouval

Abstract

Tumors are stressful environments. As tumors evolve from single mutated cancer cells into invasive malignancies they must overcome various constraints and barriers imposed by a hostile microenvironment. To achieve this, cancer cells recruit and rewire cells in their microenvironment to become pro-tumorigenic. We propose that chaperones are vital players in this process, and that activation of stress responses helps tumors adapt and evolve into aggressive malignancies, by enabling phenotypic plasticity in the tumor microenvironment (TME). In this chapter we will review evidence supporting non-cancer-cell-autonomous activity of chaperones in human patients and mouse models of cancer, discuss the mechanisms by which this non-cell-autonomous activity is mediated and provide an evolutionary perspective on the basis of this phenomenon.

Keywords

HSF1 · Chaperones · Tumor microenvironment · Cancer-associated fibroblasts · Heat shock · Stress responses · ER-stress · UPR · Cancer

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7.1 Introduction

Malignant transformation is initiated by oncogenic mutations and loss of tumor suppressor genes, causing loss of growth control. However, the resulting proliferative imbalance is usually controlled by the normal surrounding tissue, thereby suppressing tumorigenesis (Bissell and Hines 2011). Tumors form when cancer cells succeed to distort local tissue homeostasis and recruit normal cells to support their sustained proliferation and evasion of immune surveillance. These non-transformed cells protect the cancer cells and support them by creating a pro-tumorigenic tumor microenvironment (TME) or tumor stroma (Fig. 7.1a). The TME comprises a variety of cell types, including lymphocytes, macrophages, neutrophils, fibroblasts, and endothelial cells as well as extracellular matrix (ECM) components (Hanahan and Weinberg 2011; Hance et al. 2014; Place et al. 2011). Endothelial cells give rise to the neoangiogenic vasculature, recruit immune cells, and modulate cancer cell dissemination and metastasis. Neutrophils and lymphocytes can mount cancer cell-killing responses or release pro-inflammatory factors that stimulate tumor progression (Hanahan and Coussens 2012; Sagiv et al. 2015). Cancer-associated fibroblasts (CAF), which are perhaps the most abundant cells in the TME of carcinomas (Hanahan and Weinberg 2011) support cancer cells through secretion of ECM, chemokines and

M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_7

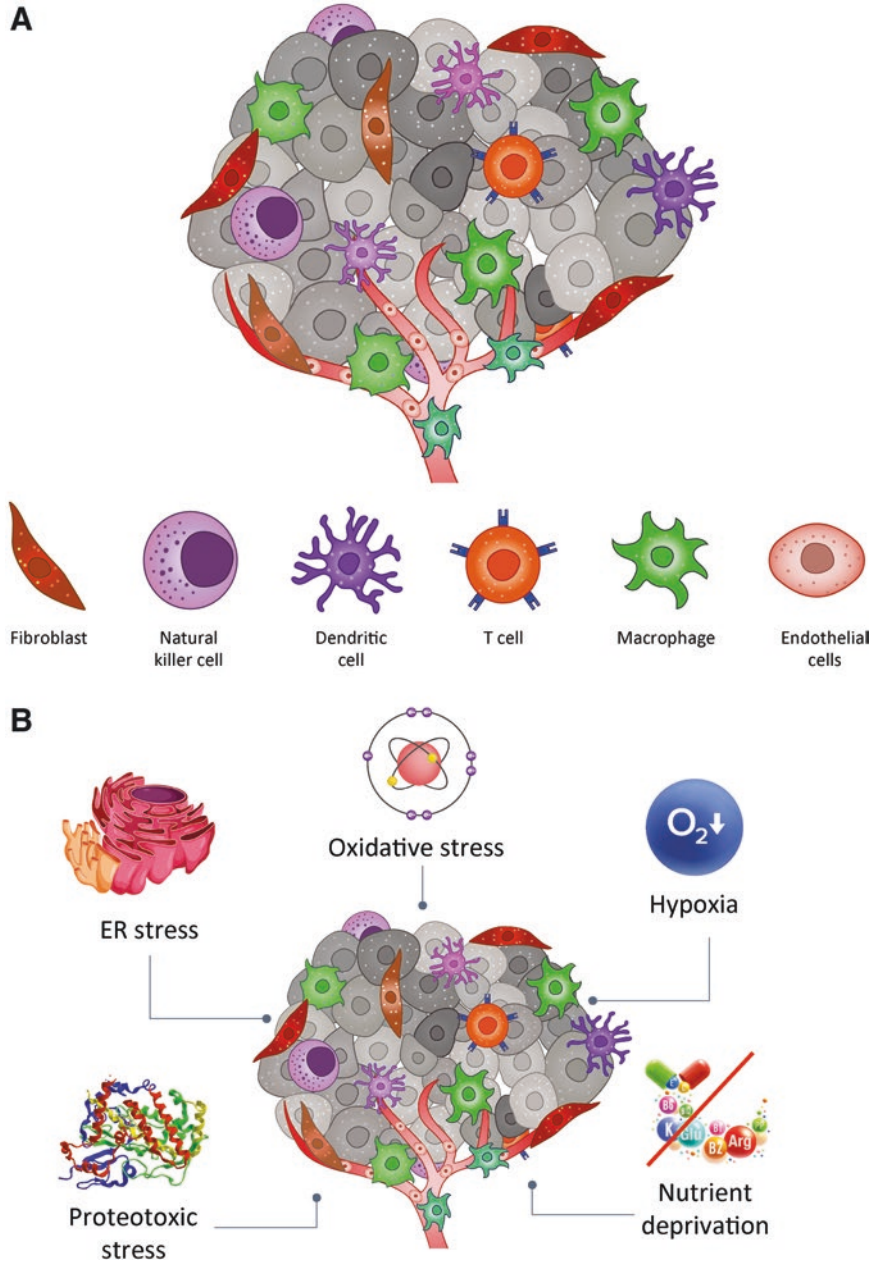

Fig. 7.1 Tumors are stressful microenvironments. (**a**) Cancer cells are depicted in shades of grey, different cell types interacting with cancer cells in the tumor microenvironment are highlighted in colors as detailed. (**b**) Stress conditions inherent to the tumor microenvironment are shown

cytokines (Alspach et al. 2014; Coppe et al. 2008; Erez et al. 2010; Finak et al. 2008). CAFs also promote the recruitment of tumor-associated macrophages (TAMs), with which they engage in a reciprocal relationship that promotes malignancy (Cirri and Chiarugi 2011; Comito et al. 2014; Erez et al. 2010). TAMs further stimulate angiogenesis, enhance cancer cell migration and invasion, and suppress anti-tumor immunity (Qian and Pollard 2010).

Throughout evolution, stress responses have helped cells and organisms survive in harsh conditions and overcome population bottlenecks. Rapidly evolving tumors are exposed to oxidative stress, DNA damage, metabolic stress, hypoxia and low pH (Fig. 7.1b; (Leprivier et al. 2015)). Moreover, they are exposed to population bottlenecks imposed by natural steps of tumor progression such as invasion and metastasis, as well as by external forces such as surgery and drug treatments. Under these extreme conditions cells of the TME and cancer cells constantly communicate with one another via secreted factors such as cytokines, chemokines, growth factors, and proteases (Witz 2008). This ongoing cell-to-cell communication helps cancer cells adapt to the stressful environment by modifying signalling pathways and reprogramming normal neighbouring cells. Cancer cells need a means of adapting their own protein machinery to function under stressful conditions and to signal to the TME to enable survival and metastasis. One way of surviving under extreme conditions is via activation of molecular chaperones.

Molecular chaperones or heat-shock proteins (HSPs) are a large family of highly conserved proteins traditionally known to be involved in protein folding, protein trafficking and assembly/disassembly of oligomeric structures. Under stress conditions they facilitate correct folding and prevent toxic protein aggregation (Hartl et al. 2011). Chaperones can be found in various subcellular and extra-cellular localizations, such as the nucleus, cytosol, endoplasmic reticulum (ER), mitochondria, lysosomes, cell-surface and blood (Henderson 2010). Depending on their localization chaperones can play important roles in inter and intracellular signalling. They interact with transcription factors, hormone receptors and kinases (Whitesell and Lindquist 2005). They integrate a wide range of cellular signalling pathways which help cells adapt to extreme environments (Maguire et al. 2002).

In tumors, chaperones promote the survival of cancer cells in the harsh tumor microenvironment. Master regulators of cytosolic chaperones such as Heat-shock factor 1 (HSF1), and ER chaperones such as XBP1 were shown to be associated with poor patient outcome and to promote cancer in mice (Romero-Ramirez et al. 2004; Santagata et al. 2011). The pro-tumorigenic activity of master regulators of stress responses such as HSF1 and XBP1 is mediated in part by activation of chaperones. Importantly however, these transcription factors activate a large repertoire of cancer-specific targets, and their activity in cancer is distinct from the typical stress-induced activity (Chen et al. 2014; Mendillo et al. 2012; Scherz-Shouval et al. 2014). Cancer cell-autonomous activities are discussed in other chapters of this book. Here we focus on the non-cancer-cell-autonomous activity of stress-activated transcription factors and chaperones and on the various ways by which they modulate the TME (Fig. 7.2; Table 7.1).

7.2 Chaperones in Human Cancer

Increased expression of chaperones is a common feature of various human cancers. Heat-shock protein 70 (Hsp70) is overexpressed in most human cancers, including breast, colon, liver, prostate and acute myeloid leukaemia (AML) (Sherman and Gabai 2015; Steiner et al. 2006). Importantly however, the correlation between its expression and patient outcome is mixed and is cancer type specific: in gastric cancer Hsp70 expression has no prognostic value, and in renal and oesophageal cancer increased levels of Hsp70 are associated with better disease outcome (Maehara et al. 2000; Sherman and Gabai 2015; Shiozaki et al. 2000). Heat-shock protein 90 (Hsp90) is overexpressed in various human cancers as well (Ciocca et al. 2013; Pick et al. 2007), and has been a long sought-after therapeutic target in cancer. To date, 18 Hsp90 inhibitors have entered the clinic, of which 5 are still in active clinical trials. Unfortunately, none of these inhibitors have been FDA approved yet (Yuno et al. 2018). The main hindrance in developing cancer-selective Hsp90 inhibitors is the dependence of normal cells on Hsp90 for sur-

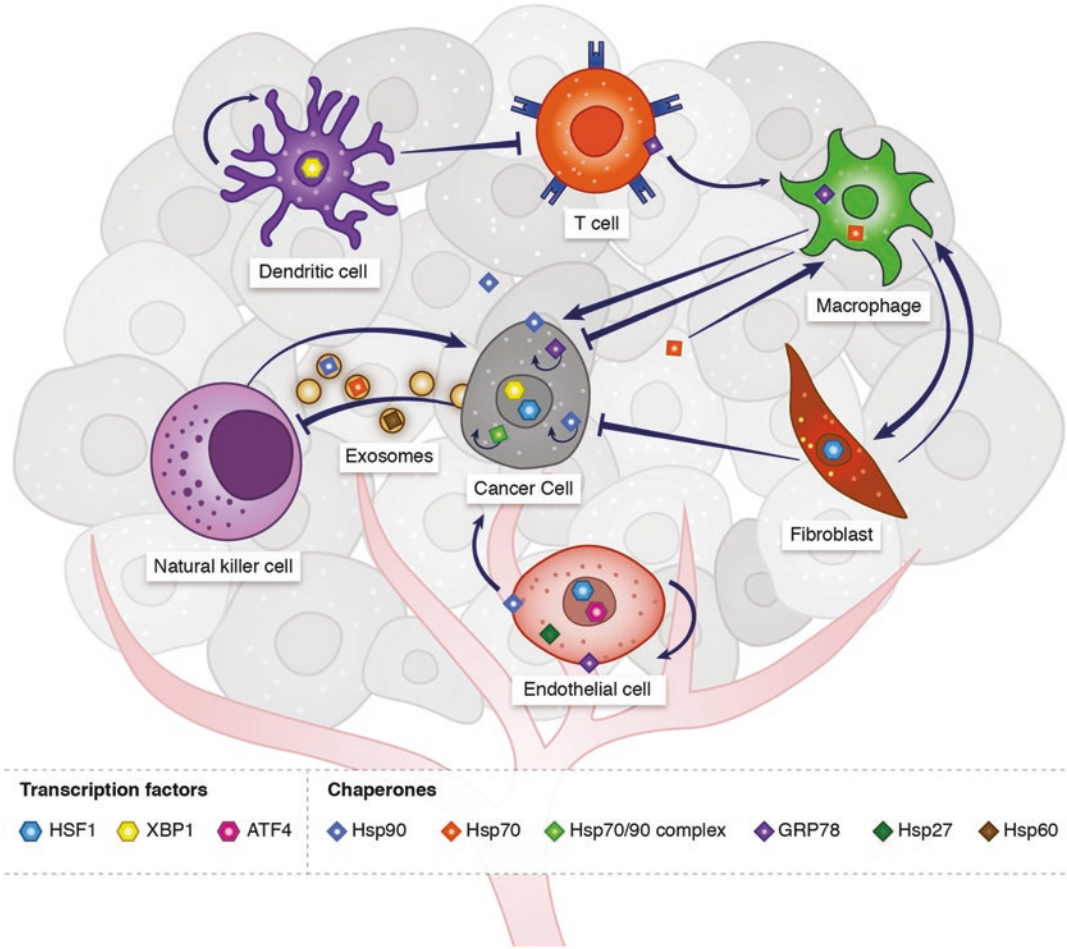

Fig. 7.2 Chaperones mediate cell-cell interactions in the tumor microenvironment. Signaling pathways mediated by stress-activated transcription factors and chaperones are presented. (See Table 7.1 for details and references)

vival, and the fact that inhibition of Hsp90 leads to activation of HSF1 (Whitesell et al. 2014; Yuno et al. 2018). Both could potentially be overcome by applying low-level inhibition of Hsp90 in combination with chemotherapy or hormonal therapy (Whitesell et al. 2014). The ER Hsp70 chaperone glucose regulated protein 78 (GRP78), also known as the immunoglobulin heavy chain binding protein (BiP), is induced under stress conditions such as glucose depletion, anoxia, acidosis and ER stress. In patients GRP78 is thought to be involved in the development of castration-resistant prostate cancer and increased levels of it are associated with disease recurrence in prostate cancer (Daneshmand et al. 2007; Pootrakul et al. 2006). Not only chaperones, but also the factors driving their expression are activated in human cancer. Activation of HSF1 both in cancer cells and in CAFs is associated with poor patient outcome in a variety of human carcinomas (Liao et al. 2015; Mendillo et al. 2012; Scherz-Shouval et al. 2014). Overexpression of activated XBP1 correlates with poor prognosis in glioblastoma (Avril et al. 2017; Obacz et al. 2017; Pluquet et al. 2013; Rubio-Patino et al. 2018), and in luminal/ER+ as well as triple-negative breast cancer (TNBC) (Cancer Genome Atlas Network 2012; Chen et al. 2014). Another arm of the unfolded protein response (UPR), that of PKR-like ER kinase (PERK) is upregulated in human breast ductal carcinomas in situ (DCIS), where PERK phosphorylation is higher in DCIS tissue than normal breast (Avivar-Valderas et al. 2011).

Table 7.1 Non-cell-autonomous signaling mediated by stress-activated transcription factors and chaperones in the tumor microenvironment

Molecule	Cell of origin	Target cell	Effect	References
HSP70/90 complex	Cancer	Cancer	Regulates MMP2 activation-promotes tumor invasion	Eustace et al. (2004) and Sims et al. (2011)
eHSP90	Cancer	Cancer	Interacts with client proteins such as HER2 and regulates signaling	Hance et al. (2014) and Sidera et al. (2008)
HSF1	Cancer	Cancer	Promotes proliferation, invasion and migration	Mendillo et al. (2012)
GRP78	Cancer	Cancer	Activates the UPR	Obacz et al. (2017)
eHSP90	Cancer	Fibroblast	Regulates MMP activation-promotes tumor invasion	Correia et al. (2013)
eHSP70	Cancer	Antigen Presenting Cells (APC)	Triggers an immune response	Theriault et al. (2005) and Zhou and Binder (2014)
HSP60, HSP70, HSP90 in exosomes	Cancer	Natural Killer (NK) Cells	Trigger NK migration and cytosolic activation- anti-tumor effects	Gastpar et al. (2005)
IRE1/XBP1	Cancer	Macrophages	Activate innate immune responses	Martinon et al. (2010)
HSF1	Fibroblasts	Cancer	Activates a wound healing program that promotes tumor growth and survival	Scherz-Shouval et al. (2014)
HSF1	Endothelial cells	Endothelial Cells	Thermotolerance	Bagley et al. (2015)
Hsp70	Macrophages	Cancer	Promotes migration and infiltration into tumors	Gabai et al. (2016)
GRP78	Macrophages and T cells	Cancer	Leads to secretion of cancer associated cytokines	Li and Li (2012)
CHOP	Myeloid derived suppressor cells (MDSCs)	Cancer	Promotes anti-tumor immunity	Thevenot et al. (2014)
GRP78	Endothelial	Endothelial	Angiogenesis	Virrey et al. (2008) and Dong et al. (2011)
XBP1	Dendritic cell (DC)	DC/cancer	Prevents T-cell mediated anti-tumor immunity	Cubillos-Ruiz et al. (2015)
PERK/ATF4	Endothelial	Endothelial	Induce secretion of pro-angiogenic factors	Cubillos-Ruiz et al. (2017) and Wang et al. (2012)
HSP27	Endothelial	Endothelial	Promotes angiogenesis	Lee et al. (2012)
HSP90	Endothelial	Endothelial	Increases angiogenesis by regulating HIF1-alpha and VEGF signaling	Okui et al. (2011)

7.3 Mechanisms of Chaperone Mediated Tumor-Stroma Interactions

Chaperones play an important role in linking stress at the cellular level to stress in the organism. Though classically considered cell autonomous survival pathways, evidence suggesting non-cell autonomous activation of the heat shock response (HSR) and the unfolded protein response (UPR) has been accumulating. This systemic activation of proteostasis mechanisms allows a coordinated response to stress and coordinated ageing of cells in different tissues (Taylor et al. 2014; van Oosten-Hawle et al. 2013). Hijacked by cancer, it also supports the growth of tumors at the expense of

the host. Chaperones and stress-activated transcription factors in tumors exert their non-cell autonomous effects via two main routes: (1) Activated in cancer cells, they mediate intercellular communication with cells of the TME through direct secretion from cancer cells or by chaperoning of cell-surface proteins, ECM components, and secreted molecules (2) Activated in cells of the TME, they facilitate cancer-promoting activities such as immune modulation, ECM remodeling and angiogenesis.

7.4 Chaperones Are Secreted to the Extracellular Space

In addition to their canonical cytosolic activity, both Hsp70 and Hsp90 can act as cell surface and extracellular chaperones, and this activity could be tumor promoting but also anti-tumorigenic. The term 'chaperokine' was coined to describe extracellular Hsp70's dual role as a chaperone and a cytokine (Asea 2005). Extracellular Hsp70 (eHsp70) can bind to cell surface receptors such as CD91 and LOX-1 on antigen presenting cells (APC) and elicit immune responses through presentation of peptides from its chaperoned clients (Theriault et al. 2005; Zhou and Binder 2014). This antigen presenting activity is shared with other Hsps including Hsp60 and Hsp90 (Asea et al. 2000; Quintana and Cohen 2005). Such immune modulating activities could explain why, in some cancers, Hsp70 expression is correlated with better prognosis.

Extracellular Hsp90 (eHsp90) can play a tumor-repressive role through similar antigen presentation activities to those of Hsp70. However, it has many tumor-promoting effects, since it supports wound healing, tissue regeneration and cell migration (Hance et al. 2014). Selective inhibition of eHsp90 activity by cell impermeant molecules results in reduced cancer cell motility and invasion (Tsutsumi et al. 2008). But how is it secreted and what is the mechanism by which it promotes migration and invasion? eHsp90 release is triggered by necrosis, disruption of membrane integrity, growth factors and a variety of stress factors including DNA damage, oxidative stress, heat stress, hypoxia and exposure to chemotherapy (Hance et al. 2014). In the extracellular space eHsp90 serves as a major regulator of metalloprotease (MMP) activity (Correia et al. 2013; Eustace et al. 2004). MMP-2 is a client of the Hsp70/Hsp90 organizing protein (HOP) complex and the stabilizing interaction of these chaperones with MMP-2 promotes tumor invasion (Eustace et al. 2004; Sims et al. 2011). In advanced stage gastric cancer patients Hsp90 expression is significantly correlated with MMP-9 expression, and associated with poor prognosis (Wang et al. 2013). Secreted eHsp90 also promotes mammary epithelial invasion through its activation of MMP-3 (Correia et al. 2013). By controlling the activity of MMPs, eHsp90 indirectly controls the structure and composition of the ECM. eHsp90 also regulates signalling through its interaction with cell surface receptors such as low-density lipoprotein receptor-related protein 1 (LRP1) and HER2 (Hance et al. 2014; Sidera et al. 2008).

7.5 Chaperones Mediate Cell-Cell Communication via Exosomes

Chaperones were spotted on the surface of cancer cells and in extracellular domains more than a decade ago (Shin et al. 2003; Tsutsumi et al. 2008), but the mechanism by which they are exported from cells was not clear. A growing body of evidence suggests that at least part of the extracellular activity of chaperones is mediated through exosomes. Exosomes are nano-extracellular vesicles released by different types of mammalian cells. They contain protein, RNA and DNA. In cancer, exosomes are critical mediators of the communication between cancer cells and cells of the TME (Becker et al. 2016). Under stress conditions and in response to drug treatment, the secretion of Hsp60, Hsp70 and Hsp90 from cancer cells via exosomes is increased (Lv et al. 2012), stimulating migratory and cytolytic activity of natural killer (NK) cells and thereby serving as anti-tumor agents (Gastpar et al. 2005). Beneficial effects were also reported for small

Hsps (Hsp27 and Hsp20) secreted through exosomes in various pathological conditions (Reddy et al. 2018), and Hsp20 has been implicated in exosome biogenesis (Wang et al. 2016). Notably, intercellular transmission of chaperones mediated by exosomes was shown to maintain and improve proteostasis in recipient cells (Takeuchi et al. 2015). Though this hasn't been reported in cancer, one could envision how such non-cell-autonomous maintenance of organismal proteostasis is subverted to promote the survival of tumors.

7.6 Chaperones Are Activated in Cells of the TME

With the growing understanding that cells of the TME play important roles in cancer, an increasing number of studies have recently shown activation of stress-induced transcription factors such as HSF1 and XBP1 and chaperones including Hsp70, Hsp90 and GRP78 in cells of the TME. HSF1 is activated in cancer cells as well as in fibroblasts and endothelial cells, and in each cell type it drives a distinct transcriptional program. In cancer cells HSF1 promotes proliferation, invasion and migration (Mendillo et al. 2012). In fibroblasts HSF1 drives a wound-healing CAF signature, promoting the growth and malignant properties of adjacent cancer cells (Ferrari et al. 2019; Scherz-Shouval et al. 2014). In tumor-associated endothelial cells exposed to thermal treatment, HSF1 drives classic thermotolerance, leading to decreased transport of therapeutic agents in mouse tumors (Bagley et al. 2015). How does the same transcription factor drive different transcriptional programs in different cell types and different contexts? Possibly epigenetic alterations alter promoter accessibility. Alternatively the combination of stresses in the tumor – hypoxia, nutrient deprivation, genotoxic and proteotoxic stress all together leads to activation of multiple stress responses, resulting in different transcriptional outputs in different cell types in the TME.

Nevertheless, evidence for activation of canonical chaperones in the TME is accumulating as well. In particular, Hsp70 activation in macrophages promotes their migration and infiltration into tumors, and inhibition of this activity by a pharmacological inhibitor (JG-98) or by knockout of Hsp70 in the stroma profoundly affects tumor growth in mice (Gabai et al. 2016). The ER Hsp70 GRP78 is also activated in macrophages, as well as on the surface of T-cells, in response to stress, and regulates the activity of several cancer-associated cytokines, such as interleukin-6 (IL-6), macrophage migration inhibitory factor (MIF), transformed growth factor β (TGF-β) and interleukin-10 (IL-10) (Mendillo et al. 2012). IL-6 independent proinflammatory conditioning of macrophages by cancer cells was shown in a pathway termed transmissible ER-stress (TERS), where macrophages cultured in conditioned medium from ER-stressed cancer cells become activated, and themselves undergo ER stress with the up-regulation of *Grp78*, *Gadd34*, *Chop,* and *Xbp-1*splicing (Mahadevan et al. 2011). GRP78 is also expressed on the surface of endothelial cells, where it promotes tumor vascularization and angiogenesis (Virrey et al. 2008). Its overexpression drives resistance to anti-angiogenic therapy in models of glioblastoma (Virrey et al. 2008), whereas loss of GRP78 in endothelial cells supresses tumor growth and angiogenesis in a mouse model of melanoma (Dong et al. 2011).

GRP78 controls all 3 arms of the UPR through its binding to activating transcription factor 6 (ATF6), PERK, and inositol-requiring enzyme 1 (IRE1) (Obacz et al. 2017). Upon ER-stress GRP78 releases these proteins, and the UPR is activated. All three arms of the UPR have been linked to cancer, mostly through activation in cancer cells. The IRE1/XBP1 arm has been known to regulate innate immune responses in macrophages in response to pathogen-induced TLR activation (Martinon et al. 2010) and was suspected to do so in tumors as well. Recently an immunomodulatory role was shown for IRE1/XBP1 in dendritic cells (DC) in ovarian cancer (Cubillos-Ruiz et al. 2015). Activation of XBP1 in tumor-associated DCs disrupts their homeostasis and prevents T cell mediated anti-tumor immunity. Silencing of XBP1 in DCs reversed this process and prolonged survival of

ovarian cancer-bearing mice (Cubillos-Ruiz et al. 2015). The cellular stress sensor CHOP is also activated in the TME, where it modulates anti-tumor immunity. Deletion of CHOP in Myeloid-derived suppressor cells (MDSCs) leads to induction of IL-6, resulting in T cell proliferation and anti-tumor responses in tumor-bearing mice (Thevenot et al. 2014). Thus, activation of chaperones in cells of the TME does not merely promote survival of the cells in which they are activated, but actually drives specific cancer phenotypes, thereby supporting survival of the tumor in a non-cell-autonomous manner.

7.7 Chaperones Play a Role in Angiogenesis

Starvation for oxygen leading to hypoxia triggers angiogenesis. Cancer cells sense hypoxia through HIF1-alpha, and trigger angiogenesis by upregulation of vascular endothelial growth factor (VEGF). The UPR plays a critical role in this process, triggered by exposure of endothelial cells to different types of stress such as hypoxia, low pH and glucose deprivation. The PERK/ATF4 axis promotes pro-angiogenic factors including VEGF, FGF-2 and IL-6 while decreasing angiogenic inhibitors including THBS1, CXCL14 and CXCL10 mRNA (Cubillos-Ruiz et al. 2017; Wang et al. 2012). XBP1 and ATF4 can directly bind and transactivate the promoter of VEGFA (Pereira et al. 2010), and all three arms of the UPR (IRE1, ATF6 and PERK) can induce endothelial cells to secrete VEGF (Karali et al. 2014). Several reports imply that HSPs may also play a role in angiogenesis (Calderwood and Gong 2016). Hsp27 secreted from endothelial cells regulates angiogenesis via direct binding to VEGF (Lee et al. 2012). Hsp90 can also increase angiogenesis by regulating HIF1-alpha/VEGF signalling, and inhibition of Hsp90 induces HIF1-alpha and VEGF degradation (Okui et al. 2011). To continue positively regulating angiogenesis endothelial cells secrete Hsp90 to the TME where it acts through chaperoning of MMP2 (Song et al. 2010).

7.8 Concluding Remarks

Cancer cells co-evolve with cells of the TME. The balance between tumor promoting and tumor repressive activities of the TME dictates whether tumors will progress, invade, and metastasize, or whether they remain dormant. Normal cells will naturally repress cancer-promoting phenotypes, and must be massively rewired to do otherwise. How do chaperones play into this rewiring? As mechanisms of stromal rewiring are being discovered, the paradoxical role of chaperones in cancer unravels. Chaperones are activated in cancer-associated fibroblasts, macrophages, dendritic cells, NK cells, MDSCs, and endothelial cells. As crucial mediators of cell survival they are potentially ideal targets for anti-cancer therapy – they promote invasion, angiogenesis, ECM remodeling, immune evasion, and drug resistance. Yet they also activate anti-cancer immunity. Moreover, inhibition of chaperone activity could lead to protein aggregation and related pathological conditions. Deeper mechanistic study of the pathways activating chaperones in different types of cancer – the regulators, the clients, the targets and the balance between cytosolic and extracellular activities are crucial for our understanding of the interplay between cancer, stress and evolution, and for successful translation of this knowledge into useful therapies.

Acknowledgements N.G. is supported by the Israel cancer research foundation. O.L.G. is supported by the Minerva foundation and the Thompson family foundation. R.S.S is supported by the Israel Science Foundation (grants No. 401/17 and 1384/1), the European Research Council (ERC grant agreement 754320), the Laura Gurwin Flug Family Fund, the Peter and Patricia Gruber Awards, the Comisaroff Family Trust, the Estate of Annice Anzelewitz, and the Estate of Mordecai M. Roshwal. R.S.S is the incumbent of the Ernst and Kaethe Ascher Career Development Chair in Life Sciences.

References

Alspach E, Flanagan KC, Luo X, Ruhland MK, Huang H, Pazolli E, Donlin MJ, Marsh T, Piwnica-Worms D, Monahan J et al (2014) p38MAPK plays a crucial role in stromal-mediated tumorigenesis. Cancer Discov 4:716–729

Asea A (2005) Stress proteins and initiation of immune response: chaperokine activity of hsp72. Exerc Immunol Rev 11:34–45

Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442

Avivar-Valderas A, Salas E, Bobrovnikova-Marjon E, Diehl JA, Nagi C, Debnath J, Aguirre-Ghiso JA (2011) PERK integrates autophagy and oxidative stress responses to promote survival during extracellular matrix detachment. Mol Cell Biol 31:3616–3629

Avril T, Vauleon E, Chevet E (2017) Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. Oncogene 6:e373

Bagley AF, Scherz-Shouval R, Galie PA, Zhang AQ, Wyckoff J, Whitesell L, Chen CS, Lindquist S, Bhatia SN (2015) Endothelial thermotolerance impairs nanoparticle transport in tumors. Cancer Res 75:3255–3267

Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D (2016) Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. Cancer Cell 30:836–848

Bissell MJ, Hines WC (2011) Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med 17:320–329

Calderwood SK, Gong J (2016) Heat shock proteins promote cancer: It's a protection racket. Trends Biochem Sci 41:311–323

Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70

Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y et al (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature 508:103–107

Ciocca DR, Arrigo AP, Calderwood SK (2013) Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. Arch Toxicol 87:19–48

Cirri P, Chiarugi P (2011) Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res 1:482–497

Comito G, Giannoni E, Segura CP, Barcellos-de-Souza P, Raspollini MR, Baroni G, Lanciotti M, Serni S, Chiarugi P (2014) Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. Oncogene 33:2423–2431

Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6:2853–2868

Correia AL, Mori H, Chen EI, Schmitt FC, Bissell MJ (2013) The hemopexin domain of MMP3 is responsible for mammary epithelial invasion and morphogenesis through extracellular interaction with HSP90beta. Genes Dev 27:805–817

Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, Zhang S, Bettigole SE, Gupta D, Holcomb K et al (2015) ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell 161:1527–1538

Cubillos-Ruiz JR, Bettigole SE, Glimcher LH (2017) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell 168:692–706

Daneshmand S, Quek ML, Lin E, Lee C, Cote RJ, Hawes D, Cai J, Groshen S, Lieskovsky G, Skinner DG et al (2007) Glucose-regulated protein GRP78 is upregulated in prostate cancer and correlates with recurrence and survival. Hum Pathol 38:1547–1552

Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, Jhaveri N, Zhu G, Ye R, Liu Z et al (2011) A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res 71:2848–2857

Erez N, Truitt M, Olson P, Arron ST, Hanahan D (2010) Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-dependent manner. Cancer Cell 17:135–147

Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G et al (2004) Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. Nat Cell Biol 6:507–514

Ferrari N, Ranftl R, Chicherova I, Slaven ND, Moeendarbary E, Farrugia AJ, Lam M, Semiannikova M, Westergaard MCW, Tchou J et al (2019) Dickkopf-3 links HSF1 and YAP/TAZ signalling to control aggressive behaviours in cancer-associated fibroblasts. Nat Commun 10:130

Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, Chen H, Omeroglu G, Meterissian S, Omeroglu A et al (2008) Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14:518–527

Gabai VL, Yaglom JA, Wang Y, Meng L, Shao H, Kim G, Colvin T, Gestwicki J, Sherman MY (2016) Anticancer effects of targeting Hsp70 in tumor stromal cells. Cancer Res 76:5926–5932

Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroeder JA, Multhoff G (2005) Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res 65:5238–5247

Hanahan D, Coussens LM (2012) Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 21:309–322

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Hance MW, Nolan KD, Isaacs JS (2014) The double-edged sword: conserved functions of extracellular hsp90 in wound healing and cancer. Cancers (Basel) 6:1065–1097

Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475:324–332

Henderson B (2010) Integrating the cell stress response: a new view of molecular chaperones as immunological and physiological homeostatic regulators. Cell Biochem Funct 28:1–14

Karali E, Bellou S, Stellas D, Klinakis A, Murphy C, Fotsis T (2014) VEGF signals through ATF6 and PERK to promote endothelial cell survival and angiogenesis in the absence of ER stress. Mol Cell 54:559–572

Lee YJ, Lee HJ, Choi SH, Jin YB, An HJ, Kang JH, Yoon SS, Lee YS (2012) Soluble HSPB1 regulates VEGF-mediated angiogenesis through their direct interaction. Angiogenesis 15:229–242

Leprivier G, Rotblat B, Khan D, Jan E, Sorensen PH (2015) Stress-mediated translational control in cancer cells. Biochim Biophys Acta 1849:845–860

Li Z, Li Z (2012) Glucose regulated protein 78: a critical link between tumor microenvironment and cancer hallmarks. Biochim Biophys Acta 1826:13–22

Liao Y, Xue Y, Zhang L, Feng X, Liu W, Zhang G (2015) Higher heat shock factor 1 expression in tumor stroma predicts poor prognosis in esophageal squamous cell carcinoma patients. J Transl Med 13:338

Lv LH, Wan YL, Lin Y, Zhang W, Yang M, Li GL, Lin HM, Shang CZ, Chen YJ, Min J (2012) Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro. J Biol Chem 287:15874–15885

Maehara Y, Oki E, Abe T, Tokunaga E, Shibahara K, Kakeji Y, Sugimachi K (2000) Overexpression of the heat shock protein HSP70 family and p53 protein and prognosis for patients with gastric cancer. Oncology 58:144–151

Maguire M, Coates AR, Henderson B (2002) Chaperonin 60 unfolds its secrets of cellular communication. Cell Stress Chaperones 7:317–329

Mahadevan NR, Rodvold J, Sepulveda H, Rossi S, Drew AF, Zanetti M (2011) Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. Proc Natl Acad Sci U S A 108:6561–6566

Martinon F, Chen X, Lee AH, Glimcher LH (2010) TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. Nat Immunol 11:411–418

Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, Fraenkel E, Ince TA, Whitesell L, Lindquist S (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. Cell 150:549–562

Obacz J, Avril T, Rubio-Patino C, Bossowski JP, Igbaria A, Ricci JE, Chevet E (2017) Regulation of tumor-stroma interactions by the unfolded protein response. FEBS J 286(2):279–296

Okui T, Shimo T, Hassan NM, Fukazawa T, Kurio N, Takaoka M, Naomoto Y, Sasaki A (2011) Antitumor effect of novel HSP90 inhibitor NVP-AUY922 against oral squamous cell carcinoma. Anticancer Res 31:1197–1204

Pereira ER, Liao N, Neale GA, Hendershot LM (2010) Transcriptional and post-transcriptional regulation of proangiogenic factors by the unfolded protein response. PLoS One 5(9):pii: e12521

Pick E, Kluger Y, Giltnane JM, Moeder C, Camp RL, Rimm DL, Kluger HM (2007) High HSP90 expression is associated with decreased survival in breast cancer. Cancer Res 67:2932–2937

Place AE, Jin Huh S, Polyak K (2011) The microenvironment in breast cancer progression: biology and implications for treatment. Breast Cancer Res 13:227

Pluquet O, Dejeans N, Bouchecareilh M, Lhomond S, Pineau R, Higa A, Delugin M, Combe C, Loriot S, Cubel G et al (2013) Posttranscriptional regulation of PER1 underlies the oncogenic function of IREalpha. Cancer Res 73:4732–4743

Pootrakul L, Datar RH, Shi SR, Cai J, Hawes D, Groshen SG, Lee AS, Cote RJ (2006) Expression of stress response protein Grp78 is associated with the development of castration-resistant prostate cancer. Clin Cancer Res 12:5987–5993

Qian BZ, Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. Cell 141:39–51

Quintana FJ, Cohen IR (2005) Heat shock proteins as endogenous adjuvants in sterile and septic inflammation. J Immunol 175:2777–2782

Reddy VS, Madala SK, Trinath J, Reddy GB (2018) Extracellular small heat shock proteins: exosomal biogenesis and function. Cell Stress Chaperones 23:441–454

Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H, Mori K, Glimcher LH, Denko NC, Giaccia AJ et al (2004) XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. Cancer Res 64:5943–5947

Rubio-Patino C, Bossowski JP, De Donatis GM, Mondragon L, Villa E, Aira LE, Chiche J, Mhaidly R, Lebeaupin C, Marchetti S et al (2018) Low-protein diet induces IRE1alpha-dependent anticancer immunosurveillance. Cell Metab 27:828–842.e7

Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, Damti P, Lumbroso D, Polyansky L, Sionov RV et al (2015) Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep 10:562–573

Santagata S, Hu R, Lin NU, Mendillo ML, Collins LC, Hankinson SE, Schnitt SJ, Whitesell L, Tamimi RM, Lindquist S et al (2011) High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor

prognosis in breast cancer. Proc Natl Acad Sci U S A 108:18378–18383

Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, Beck AH, Dias-Santagata D, Koeva M, Stemmer SM, Whitesell L et al (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. Cell 158:564–578

Sherman MY, Gabai VL (2015) Hsp70 in cancer: back to the future. Oncogene 34:4153–4161

Shin BK, Wang H, Yim AM, Le Naour F, Brichory F, Jang JH, Zhao R, Puravs E, Tra J, Michael CW et al (2003) Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. J Biol Chem 278:7607–7616

Shiozaki H, Doki Y, Kawanishi K, Shamma A, Yano M, Inoue M, Monden M (2000) Clinical application of malignancy potential grading as a prognostic factor of human esophageal cancers. Surgery 127:552–561

Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E (2008) A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. J Biol Chem 283:2031–2041

Sims JD, McCready J, Jay DG (2011) Extracellular heat shock protein (Hsp)70 and Hsp90alpha assist in matrix metalloproteinase-2 activation and breast cancer cell migration and invasion. PLoS One 6:e18848

Song X, Wang X, Zhuo W, Shi H, Feng D, Sun Y, Liang Y, Fu Y, Zhou D, Luo Y (2010) The regulatory mechanism of extracellular Hsp90{alpha} on matrix metalloproteinase-2 processing and tumor angiogenesis. J Biol Chem 285:40039–40049

Steiner K, Graf M, Hecht K, Reif S, Rossbacher L, Pfister K, Kolb HJ, Schmetzer HM, Multhoff G (2006) High HSP70-membrane expression on leukemic cells from patients with acute myeloid leukemia is associated with a worse prognosis. Leukemia 20:2076–2079

Takeuchi T, Suzuki M, Fujikake N, Popiel HA, Kikuchi H, Futaki S, Wada K, Nagai Y (2015) Intercellular chaperone transmission via exosomes contributes to maintenance of protein homeostasis at the organismal level. Proc Natl Acad Sci U S A 112:E2497–E2506

Taylor RC, Berendzen KM, Dillin A (2014) Systemic stress signalling: understanding the cell non-autonomous control of proteostasis. Nat Rev Mol Cell Biol 15:211–217

Theriault JR, Mambula SS, Sawamura T, Stevenson MA, Calderwood SK (2005) Extracellular HSP70 binding to surface receptors present on antigen presenting cells and endothelial/epithelial cells. FEBS Lett 579:1951–1960

Thevenot PT, Sierra RA, Raber PL, Al-Khami AA, Trillo-Tinoco J, Zarreii P, Ochoa AC, Cui Y, Del Valle L, Rodriguez PC (2014) The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. Immunity 41:389–401

Tsutsumi S, Scroggins B, Koga F, Lee MJ, Trepel J, Felts S, Carreras C, Neckers L (2008) A small molecule cell-impermeant Hsp90 antagonist inhibits tumor cell motility and invasion. Oncogene 27:2478–2487

van Oosten-Hawle P, Porter RS, Morimoto RI (2013) Regulation of organismal proteostasis by transcellular chaperone signaling. Cell 153:1366–1378

Virrey JJ, Dong D, Stiles C, Patterson JB, Pen L, Ni M, Schonthal AH, Chen TC, Hofman FM, Lee AS (2008) Stress chaperone GRP78/BiP confers chemoresistance to tumor-associated endothelial cells. Mol Cancer Res 6:1268–1275

Wang Y, Alam GN, Ning Y, Visioli F, Dong Z, Nor JE, Polverini PJ (2012) The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. Cancer Res 72:5396–5406

Wang J, Cui S, Zhang X, Wu Y, Tang H (2013) High expression of heat shock protein 90 is associated with tumor aggressiveness and poor prognosis in patients with advanced gastric cancer. PLoS One 8:e62876

Wang Z, Chen JQ, Liu JL, Tian L (2016) Exosomes in tumor microenvironment: novel transporters and biomarkers. J Transl Med 14:297

Whitesell L, Lindquist SL (2005) HSP90 and the chaperoning of cancer. Nat Rev Cancer 5:761–772

Whitesell L, Santagata S, Mendillo ML, Lin NU, Proia DA, Lindquist S (2014) HSP90 empowers evolution of resistance to hormonal therapy in human breast cancer models. Proc Natl Acad Sci U S A 111:18297–18302

Witz IP (2008) Tumor-microenvironment interactions: dangerous liaisons. Adv Cancer Res 100:203–229

Yuno A, Lee MJ, Lee S, Tomita Y, Rekhtman D, Moore B, Trepel JB (2018) Clinical evaluation and biomarker profiling of Hsp90 inhibitors. Methods Mol Biol 1709:423–441

Zhou YJ, Binder RJ (2014) The heat shock protein-CD91 pathway mediates tumor immunosurveillance. Oncoimmunology 3:e28222

8 The Impact of the ER Unfolded Protein Response on Cancer Initiation and Progression: Therapeutic Implications

Cynthia Lebeaupin, Jing Yong, and Randal J. Kaufman

Abstract

Cellular stress induced by the accumulation of misfolded proteins in the endoplasmic reticulum (ER) activates an elaborate signalling network termed the unfolded protein response (UPR). This adaptive response is mediated by the transmembrane signal transducers IRE1, PERK, and ATF6 to decide cell fate of recovery or death. In malignant cells, UPR signalling may be required to maintain ER homeostasis and survival in the tumor microenvironment characterized by oxidative stress, hypoxia, lactic acidosis and compromised protein folding. Here we provide an overview of the ER response to cellular stress and how the sustained activation of this network enables malignant cells to develop tumorigenic, metastatic and drug-resistant capacities to thrive under adverse conditions. Understanding the complexity of ER stress responses and how to target the UPR in disease will have significant potential for novel future therapeutics.

Keywords

ATF6 · Oxidative stress · ER stress · UPR · IRE1α · XBP1 · ATF4 · PERK · Cancer · BiP · Chaperones

8.1 Adaptive Signalling for Endoplasmic Reticulum (ER) Protein Homeostasis

The endoplasmic reticulum (ER) is a highly dynamic stress-sensing organelle essential for cellular homeostasis that integrates different extracellular and intracellular stimuli to coordinate downstream translational and transcriptional responses. The ER is a major platform for secretory protein homeostasis, or proteostasis, that consists of the coordinated folding, processing and trafficking of at least a third of the proteome, coupled to quality control mechanisms. Understanding how cells ensure the conformational integrity of their proteome when challenged with acute and chronic stress is fundamental to health and disease. Research in the past decades has helped unravel the complexity of protein folding and revealed that aberrant folding and aggregation of proteins can lead to disease.

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_8

8.2 ER Protein Folding and Quality Control Mechanisms

The life cycle of a protein begins as a linear sequence of amino acids extending from the 80S ribosome. Proteins destined for intracellular organelles and secretion are co- or post- translationally translocated across the ER membrane in an unfolded state. The folding of a polypeptide in the ER allows it to acquire its native conformation and associated post-translational modifications and is often facilitated by ER resident chaperones such as immunoglobulin binding protein (BiP/GRP78), calnexin and calreticulin and enzymes including protein disulfide isomerases (PDIs) and *cis-trans* peptidyl-prolyl isomerases (PPIs). Correctly folded proteins are then transported to the Golgi compartment and subsequently sorted for trafficking to their ultimate cellular destination.

Due to the complexity of protein folding, it is the most error-prone step in gene expression (Wang and Kaufman 2016). As a result, quality-control machines engage terminally misfolded proteins for ER-associated degradation (ERAD) and/or autophagy to selectively degrade the misfolded protein or to activate the unfolded protein response (UPR). The UPR is an adaptive mechanism that re-establishes proteostasis in the ER. However, if the adaptive UPR is overwhelmed by chronic or severe ER protein misfolding and is unable to preserve ER function, the UPR activates an apoptotic response. It is believed that apoptotic cell death prevents release of misfolded non-functional proteins from the cell.

8.3 The Unfolded Protein Response (UPR)

When misfolded proteins accumulate in the ER, the cell engages the UPR to increase the ER protein-folding capacity for its needs (Fig. 8.1). The UPR is a conserved signalling network evolved to restore ER homeostasis and in metazoans involves the activation of three transmembrane sensors: (i) inositol-requiring enzyme 1 (IRE1); (ii) PKR-like ER kinase (PERK); and (iii) activating transcription factor 6 (ATF6). These ER-localized UPR signal transducers convey information about the intensity and duration of the stress stimuli through the detection of misfolded proteins in the ER and signal to the cytosol and nucleus to either restore proteostasis or induce apoptosis. Under unstressed conditions, IRE1α, PERK and ATF6 are maintained in inactive states by interaction with the luminal ER chaperone BiP. During ER stress, BiP binds to misfolded proteins promoting dissociation from the ER stress sensors, thereby permitting their activation to induce selective protein synthesis attenuation and gene transcription.

8.4 IRE1α

IRE1α is the most conserved UPR transducer, possessing catalytic serine/threonine kinase and endoribonuclease (RNase) activities. Following BiP dissociation from its ER luminal N-terminal domain, IRE1α dimerizes and oligomerizes while stimulating *trans*-autophosphorylation, inducing a conformational change that activates the cytosolic RNase domain (Han et al. 2009; Korennykh et al. 2009; Lee et al. 2008). Recent evidence also suggests that unfolded proteins may be sensed by binding directly to IRE1α and inducing an allosteric change that triggers IRE1α activation (Karagoz et al. 2017). Once ER protein homeostasis is restored, IRE1α oligomers disassemble concomitantly with IRE1α dephosphorylation to return to a basal IRE1α monomeric inactive state bound to BiP (Karagoz et al. 2017).

Activated IRE1α initiates the unconventional splicing of the mRNA encoding the X-box binding protein 1 (XBP1) and the regulated IRE1α-dependent decay (RIDD) (Han et al. 2009; Hollien and Weissman 2006; Hollien et al. 2009) of mRNAs and miRNAs. Spliced XBP1 (XBP1s) induces expression of genes that encode ER protein folding, secretion, ERAD and lipid synthesis functions (Hetz et al. 2011). Physiological RIDD activity appears to be an ancestral mechanism to ensure ER homeostasis by reducing the protein folding burden, while hyperactivated RIDD is

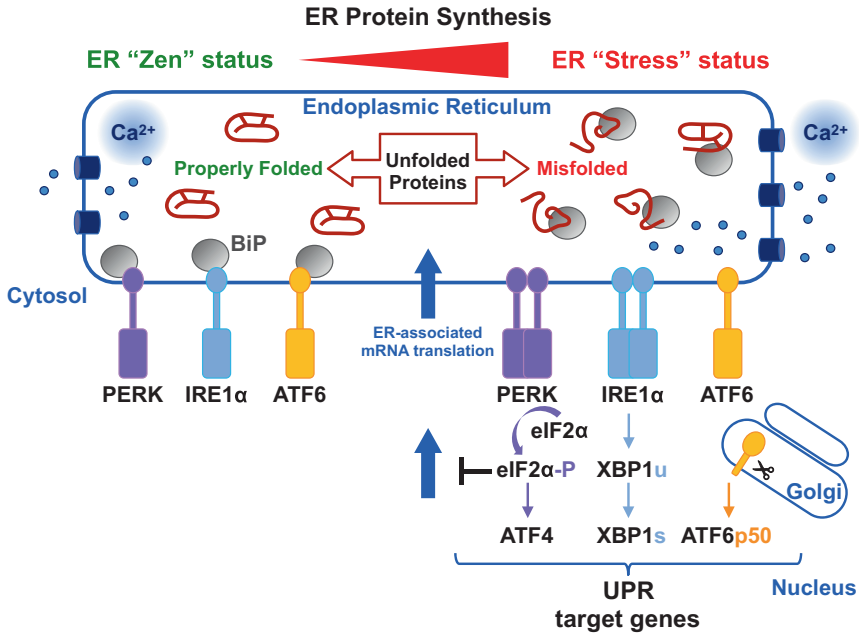

Fig. 8.1 Schematic model for ER stress and UPR activation
In the ER "Zen" status, the ER maintains protein and Ca^{2+} homeostasis. In the ER "Stress" status, accumulation of misfolded proteins induces the release of BiP from PERK, IRE1α and ATF6. These arms can be subsequently activated in a signaling cascade termed the UPR, which involves the downregulation of translation and the activation of transcription factors that regulate target genes to promote ER homeostasis and cell survival

associated with an apoptotic cellular output (Maurel et al. 2014). The mechanism controlling the switch from cytoprotective to cytotoxic RNase function remains to be identified, although it may involve oligomerization of activated IRE1α molecules (Han et al. 2009).

The IRE1α platform for signalling is described as a dynamic scaffold termed the "UPRosome" onto which regulatory components assemble to orchestrate crosstalk between the UPR and other signalling pathways (Hetz and Glimcher 2009). The direct binding of the ER chaperone HSP47 (Sepulveda et al. 2018), ERdj4/DNAJB9 (Amin-Wetzel et al. 2017), and the ER resident ER protein disulfide isomerase PDIA6 (Eletto et al. 2014; Groenendyk et al. 2014) to IRE1α may modulate its signalling behaviour. The recruitment of TRAF2 can also trigger JNK or NFκB activation, which may participate in the regulation of insulin resistance, inflammation and apoptosis (Marciniak and Ron 2006; Urano et al. 2000). IRE1α signalling may further be activated by pro-apoptotic BCL2 members BAX and BAK (Hetz et al. 2006), or negatively regulated by BI-1 (Bailly-Maitre et al. 2010; Lebeaupin et al. 2018b; Lisbona et al. 2009). Hence, the assembly of distinct adaptors and modulators on the cytosolic or luminal domains of IRE1α fine-tunes downstream signalling (Hetz et al. 2015). The physiological significance of these interactions remains to be explored.

8.5 PERK

During the early phase of ER stress, PERK is acutely activated to phosphorylate the heterotrimeric GTPase eukaryotic translation initiation factor 2 on the alpha subunit (eIF2α) at Serine residue 51 (Ser51) to attenuate protein synthesis and reduce the load on the ER to support protein folding (Kaufman 2004). Phosphorylated eIF2α halts the initiation of mRNA translation, while paradoxically increases the selective translation of numerous mRNAs that have upstream open reading frames (uORFs) in their 5′ untranslated

region, including *Atf4, Chop* and *Gadd34* (Blais et al. 2004; Harding et al. 1999). The transcription factor ATF4 transactivates a cluster of UPR target genes involved in amino acid synthesis and transport, protein synthesis and folding, autophagy, redox homeostasis, and apoptosis (Cullinan et al. 2003; Han et al. 2013; Harding et al. 2003). In particular, ATF4 induces expression of C/EBP homologous protein (CHOP) (Averous et al. 2004) and GADD34 that mediates eIF2α dephosphorylation and restores global mRNA translation (Ma and Hendershot 2003; Novoa et al. 2001). ATF4 may form heterodimers with CHOP, and transcriptional induction through ATF4 and CHOP leads to increased protein synthesis that causes oxidative stress and cell death (Han et al. 2013). Although it was originally proposed that PERK directly regulates redox homeostasis through phosphorylation of nuclear factor E2-related factor 2 (NRF2) to cause dissociation from KEAP1 permitting it to increase antioxidant gene expression, the physiological significance of this is yet to be demonstrated (Cullinan and Diehl 2004; Cullinan et al. 2003).

8.6 ATF6

ATF6 is synthesized as a type II ER-resident transmembrane protein bearing a large cytosolic N-terminal B-Zip transcriptional activation domain. Upon accumulation of unfolded proteins, ATF6 is released from BiP for trafficking to the Golgi apparatus for cleavage by the Golgi-resident proteases S1P and S2P, generating a cytosolic fragment that migrates to the nucleus to induce UPR target genes encoding functions in protein folding and ERAD (Haze et al. 1999; Nadanaka et al. 2004; Shen et al. 2002; Wu et al. 2007).

Together, the three main UPR pathways lead to activation of the major transcription factors XBP1s, ATF4, CHOP and ATF6 that govern the expression of a large range of genes encoding overlapping functions, creating a dynamic UPR network. Under conditions of chronic or severe ER stress, these UPR signalling pathways play critical roles in disease pathogenesis.

8.7 The ER Response to Cellular Stress

Despite the robustness of the ER, cells remain susceptible to various intracellular and extracellular insults that compromise protein folding or exert additional demands on the secretory pathway. Inefficient protein trafficking to the Golgi and the accumulation of misfolded proteins in the ER are responsible for the development and progression of many diseases. For example, Factor VIII expression, which is defective in the coagulation disorder hemophilia A, is limited due to unstable mRNA, polypeptide interaction with ER chaperones and inefficient transport of the primary translation product from the ER to the Golgi (Miao et al. 2004; Pipe et al. 2001). Moreover, the hallmark of many neurodegenerative diseases is the accumulation of protein aggregates. Understanding the causes of protein misfolding, protein aggregation and protein quality control is critical to developing novel therapeutic strategies for intervention in diseases of protein misfolding.

8.8 Oxidative Stress and ROS Production

Cellular stress can arise from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms that lead to organelle dysfunction. Disulfide bond formation, mediated by ER oxidases (ERO1s) and protein disulphide isomerases (PDIs), can alter the redox status of the ER (Tu and Weissman 2004). Oxidative protein folding, detoxification and mitochondrial respiration are all processes that generate ROS such as the superoxide radical (O_2-) and hydrogen peroxide (H_2O_2). If produced in excess, ROS lead to oxidative stress that damage proteins, lipids and DNA to alter cellular function and architecture and induce apoptosis (Cao and Kaufman 2014). The major pro-apoptotic factor of the UPR, CHOP, activates ERO1α transcription leading to increased ROS production and Ca^{2+}-dependent apoptosis through inositol trisphosphate receptors (IP_3R) (Li et al.

2009). Ca^{2+} released from the ER is taken up by mitochondria, which subsequently opens the permeability transition pore to release cytochrome c and activate the caspase cascade of apoptosis. Recently, IRE1α was found to physically interact with IP_3R and control mitochondrial Ca^{2+} uptake at mitochondrial-associated membranes (MAMs) (Carreras-Sureda et al. 2019).

To protect against the deleterious impact of oxidative stress, antioxidant responses exist to restore cellular redox homeostasis. Certain antioxidant genes (*Ngf*, *Ho-1*, *Txnrd1*, *xCT*, *p62*) may be enhanced by NRF2 and ATF4 cooperation (Mimura et al. 2019). Nevertheless, in pathological conditions the antioxidant response may be impaired.

8.9 Cell Death Pathways Ensuing Prolonged or Severe ER Stress

Over the past thirty years, two fundamental mechanisms by which cells die in response to ER stress were discovered: (1) ER stress directly causes oxidative stress leading to cell death (Back et al. 2009; Han et al. 2015; Malhotra et al. 2008; Song et al. 2008); and (2) Chronic ER stress paradoxically increases protein synthesis leading to proteostatic stress that causes cell death (Back et al. 2009; Han et al. 2013; Nakagawa et al. 2014; Song et al. 2008).

There is ample experimental evidence indicating that misfolded protein accumulation in the ER causes oxidative stress generated by the mitochondrial electron transport chain (ETC), and the intimate functional cooperation between ER and mitochondria is likely causal for such an observation. Mitochondria provide ATP to the ER which is essential to support ATP-dependent protein chaperone functions, such as BiP/GRP78 (Dorner et al. 1990), for protein folding and trafficking. In fact, the level of cellular ATP determines which proteins are able to transit to the cell surface (Dorner et al. 1990, 1992). In response, the ER signals mitochondria to stimulate ATP production through ER-to-mitochondria Ca^{2+} trafficking. ER stress is associated with increased Ca^{2+} trafficking through IP_3Rs on the ER membrane to enter the mitochondrial matrix (Carreras-Sureda et al. 2019; Kaufman and Malhotra 2014; Luciani et al. 2008; Yong et al. 2019). Although ER Ca^{2+} release channels have been known for decades and are relatively well-characterized (La Rovere et al. 2016), only recently has the mitochondrial Ca^{2+} uniporter (MCU) (Kwong 2017; Kwong et al. 2015; Pan et al. 2013) and the associated regulatory molecules been identified (Liu et al. 2016; Mallilankaraman et al. 2012; Sancak et al. 2013; Waldeck-Weiermair et al. 2015). Irrespective of the mechanism, Ca^{2+} trafficking from ER to mitochondria potentiates oxidative phosphorylation by activating NADH dehydrogenases (Kaufman and Malhotra 2014), which may further increase mitochondria-derived ROS production. *In vivo*, we hypothesize that a process of IP_3R-mediated Ca^{2+} release, in response to IP_3 generating hormonal receptor engagement, exacerbates ROS production due to mitochondrial Ca^{2+} overload. Supporting such a scenario, cardiomyocyte-specific *Mcu* deletion was protective in acute ischemia-reperfusion injury models (Kwong 2017; Pan et al. 2013).

Among the UPR transducers and downstream signalling molecules, the PERK-P-eIF2α pathway is intimately involved in mediating both cellular survival and death. Activated PERK phosphorylates eIF2α at Ser51, leading to rapid, reversible inhibition of mRNA translation initiation. PERK mutation in humans, and mice, is the cause of infantile onset diabetes associated with Wolcott-Rallison syndrome (Delepine et al. 2000). Experimentally, a Ser51 to Alanine (Ser51Ala) mutation introduced into the endogenous murine *eIF2α* gene generated mice that cannot phosphorylate eIF2α (Scheuner et al. 2001). Homozygous Ser51Ala mutant mice die perinatally due to hypoglycemia and defective hepatic gluconeogenesis. When fed a high fat diet (HFD) that triggers insulin resistance, heterozygous Ser51Ala mutant mice develop pancreatic β-cell failure and overt diabetes (Scheuner et al. 2005). In addition, since β-cell failure due to eIF2α-S51A mutation recapitulates all the effects of PERK deletion in mice and humans (Back et al. 2009), the findings support the model where

PERK acts through P-eIF2α to attenuate protein synthesis (mainly proinsulin) and translocation into the ER, and thereby limits ER stress-induced β-cell death. Importantly, in the setting of increased proinsulin misfolding, CHOP protein, controlled primarily via the PERK branch of the UPR pathway contributes to β-cell death associated with unsuppressed protein synthesis and increased ROS production (Han et al. 2013; Song et al. 2008). Curiously, without ER stress, sole expression of CHOP does not promote cell death, *in vitro* or *in vivo* (Han et al. 2013; Southwood et al. 2016; Wang et al. 1998). Furthermore, ChIP-Seq and mRNA-Seq did not reveal any death-promoting genes directly transcriptionally activated by CHOP (Han et al. 2013). Instead, the death-promoting effect by CHOP expression in the context of ER stress was shown to be a consequence of increased protein synthesis in stressed cells, thereby further promoting oxidative stress leading to irreparable cellular damage (Han et al. 2013; Marciniak et al. 2004; Song et al. 2008). These observations suggested that the notorious "death-promoting" role of CHOP is more accurately attributed to its transcriptional role as a master regulator of ER-oriented anabolic metabolism (Yong et al. 2016). Indeed, deletion of the *Chop* gene delays the onset of hyperglycemia in the *Akita* mouse (Oyadomari 2002) and suppresses β-cell failure in response to insulin resistance in diet-induced obese mice and the leptin receptor mutant *db/db* mice (Song et al. 2008). The significance of ROS production was demonstrated by showing that antioxidant feeding prevents β-cell death in response to ER stress (Back et al. 2009; Han et al. 2015; Hassler et al. 2015; Malhotra et al. 2008).

To summarize the role of PERK branch in the UPR, although other factors (such as inflammation pathways) are proposed to cause cell failure, analysis of mice with *eIF2α* Ser51Ala mutation or *Perk* gene deletion supports the notion that increased protein synthesis is sufficient to generate ROS through ER-to-mitochondria crosstalk (Kaufman and Malhotra 2014; La Rovere et al. 2016) that initiates cell death.

8.10 ER Stress in Tumorigenesis

UPR activation is documented in many cancer types (Wang and Kaufman 2014) (Fig. 8.2). Accumulating evidence supports the notion that UPR signalling is integral to cell transformation and tumor progression, although the mechanisms remain poorly defined. As discussed above, protein misfolding in the ER is sufficient to cause oxidative stress (Nakagawa et al. 2014), and it was proposed that ER stress-induced oxidative stress may damage DNA, and further cause somatic gene mutations (Shalapour et al. 2017), further leading to oncogene activation or tumor suppressor gene inactivation. However, once a tumor microenvironment forms, the transformed cells likely depend on UPR signalling to promote cell growth under the unfavorable conditions of the microenvironment, e.g. hypoxia, nutrient deprivation (Milani et al. 2009; Mujcic et al. 2013; Rouschop et al. 2013; Rzymski et al. 2009), and to evade immune surveillance by the adaptive immune response.

8.11 ER Stress and UPR Activation in Cancer Cells

It is incompletely understood how ER homeostasis is perturbed in cancer cells, and how UPR signalling can impact tumorigenesis. After an initiating tumorigenic event, the UPR is likely essential to protect cells from ER stress-induced cell death and thereby promotes cancer progression. Under this scenario, UPR signalling may be required to maintain ER homeostasis to prevent ROS accumulation incurred from cancer cell damage and apoptosis. For example, previous studies demonstrated a severe hypoxic microenvironment activates the PERK-eIF2α arm of the UPR, leading to increased glutathione synthesis and consequently protection against ROS produced during periods of hypoxia, which is believed to contribute to therapy-resistance of cancer cells (Rouschop et al. 2013). Epithelial-to-mesenchymal transition (EMT) and the secretory-associated senescence phenotype (SASP) are cellular processes associated with

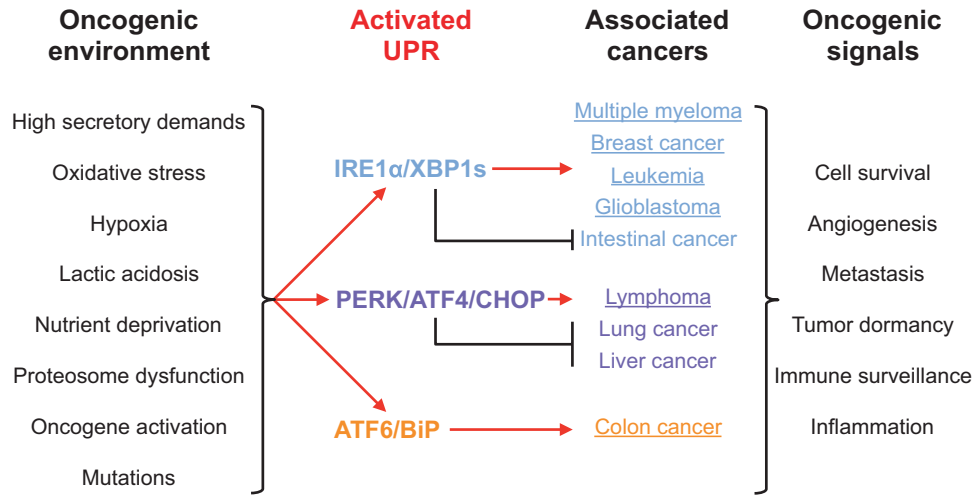

Fig. 8.2 UPR activation in the oncogenic environment Tumors frequently encounter stressors from the oncogenic environment that compromise protein folding and increase demands on the protein secretory pathway, activating the three branches of the UPR. UPR activation has been reported to promote or prevent cancer development in in a cell-specific manner. The combined outputs of the UPR can send oncogenic signals that influence cancer initiation and progression

metastasis and require increased protein secretion of cytoskeletal matrix proteins, metalloproteases and growth factors. The increased secretion can activate the UPR and thus prime the cells for preferential death upon pharmacological induction of the UPR (Denoyelle et al. 2006; Feng et al. 2014).

Cancer cells experience ER stress as identified by high level expression of UPR biomarkers (e.g., BiP/GRP78, CHOP, XBP1s and TRIB3). Some of these proteins provide protective and adaptive functions for survival of the cancer cell; most notably elevated BiP expression correlates with increased tumor grade (Dong et al. 2011). Alternatively, some of the UPR induced genes, most notably *Chop*, promote cell death. Due to the increased sensitivity of tumor cells to ER stress it may be possible to pharmacologically activate the UPR to kill tumor cells. Targeting the UPR may provide tumor-selective killing because normal cells, without basal ER stress, can mount an adaptive UPR and return to homeostasis. The majority of efforts to target the UPR in cancer are directed at compounds that activate the pro-apoptotic UPR (Flaherty et al. 2013, 2014) and will be discussed below.

8.12 The Role of IRE1 and XBP1s in Cancer

An early study discovered that IRE1α (*Ern1)* ranked 5th among 518 protein kinase genes that carry at least one driver mutation (Greenman et al. 2007), although it is unknown whether these mutations cause a gain or loss of function. In the same pathway, XBP1s was reported to drive multiple myeloma (Carrasco et al. 2007). IRE1α and XBP1 loss of function mutations were identified in tumor cells from multiple myeloma patients that were resistant to proteasome inhibitors (Hong and Hagen 2013; Leung-Hagesteijn et al. 2013). Inactivation of the IRE1α/XBP1s pathway reduced immunoglobulin (IgG) gene expression so the plasma cells dedifferentiated to preplasmablasts that were resistant to proteasome inhibition because they did not express IgG which misfolds in the ER lumen. Furthermore, XBP1 splicing was suggested to cause human triple negative breast cancers, in part through its proposed role by increasing HIF1α expression (Chen et al. 2014). Increased XBP1s expression in luminal breast cancer mediates resistance to anti-hormonal therapy by stimulating Nuclear Receptor Coactivator 3 (NCOA3) (Gupta et al.

2016). Moreover, increased XBP1 expression is commonly observed in patients with acute myeloid leukemia (~70%) and leukemia cell lines (Sun et al. 2016). Along the same line, knocking-down XBP1 expression levels increased the susceptibility of multiple myeloma cells to ER stress-inducing reagents (Mimura et al. 2012). IRE1α was also suggested to be oncogenic in glioblastoma through its RNase activity to degrade selective mRNAs by RIDD (Dejeans et al. 2012; Pluquet et al. 2013). However, recent studies suggest a dual role of IRE1α RNase in glioblastoma development, where IRE1α-mediated splicing of XBP1 mRNA is pro-tumorigenic, while IRE1α-mediated RIDD is tumor suppressive (Lhomond et al. 2018).

In contrast, loss of XBP1 promotes tumorigenesis in mouse models of intestinal cancer induced by colitis or by APC mutation (Niederreiter et al. 2013). This appears due to hyperactivation of IRE1α because tumorigenesis actually required IRE1α (Niederreiter et al. 2013). These findings indicate that the IRE1α-XBP1 pathway plays a tumor-suppressor role in cancer in the intestine. Overall, while the underlying role of IRE1α in cancer initiation is suspected, more recent findings do not fully support a uniform oncogenic role for IRE1α. To conclude, the above observations support the notion that IRE1α-XBP1 plays an oncogenic role in cancer, although the relative contribution of this pathway is highly cell-type-dependent.

8.13 The Role of PERK and ATF4 in Cancer

PERK activation promotes MYC-driven cell transformation and autophagy (Hart et al. 2012), the latter being a well-documented function of ATF4, the downstream signalling effector of PERK-P-eIF2α that initiates cell death. However, there are contradictory findings regarding whether ER stress-induced autophagy promotes tumor progression. While many studies support a cytoprotective role for autophagy in cancer cells (Cubillos-Ruiz et al. 2017; Ma et al. 2014; Ogata et al. 2006), others indicate that autophagy is important for cancer inhibition. For example, Cerezo et al. reported that BiP inhibition by the thiazole benzenesulfonamide HA15 exerts its cancer cell killing effect by causing ER stress to activate autophagy and apoptosis in a manner that requires the apoptosis factor CHOP (Cerezo et al. 2016). Similarly, other independent studies reported that *Chop* gene deletion increases K-Ras^{G12V} driven mouse lung cancer (Huber et al. 2013). In addition, *Chop* deletion increases hepatocellular carcinoma in a mouse model of hepatocyte-specific misfolding of urokinase (*MUP-uPA* transgenic mice) (Huber et al. 2013; Nakagawa et al. 2014), which is consistent with CHOP's role in apoptosis induced by ER stress. It is unknown if CHOP plays a tumor-suppressor role in human tumors, although missense mutations in the *Chop* gene were reported in human lung adenocarcinoma (Kan et al. 2010).

8.14 The Role of ATF6 and BiP/GRP78 in Cancer

Some studies suggest that ATF6α promotes tumorigenesis (Arai et al. 2006), possibly by its downstream effector ER chaperone, BiP. Elevated BiP expression is associated with benefits favoring cancer cell survival (Fu et al. 2007; Pyrko et al. 2007) and can prevent caspase activation (Reddy et al. 2003).

Inhibition of cell division due to G0/G1 cell cycle arrest and entry into quiescence are characteristic of cancer cell dormancy (Aguirre-Ghiso 2007). A main reason for cancer recurrence after radio- and chemotherapies is reactivation of dormant cells (Paez et al. 2012). There also appears to be a link between UPR activation and tumor dormancy as ATF6 is highly expressed in recurrent tumors (Ginos et al. 2004) and correlates with poor prognosis for colorectal cancer (Lin et al. 2007). ATF6 expression is elevated in metastatic lesions compared to the primary tumor (Ramaswamy et al. 2001). These findings suggest that ATF6 may be constitutively active in dormant cells, such as human squamous cell carcinoma (SCC) (Schewe and Aguirre-Ghiso 2008), but studies need to confirm this notion. Knock-down

of ATF6 in SCC reduces cancer cell survival and tumor growth via downregulation of adaptive pathways, such as mTOR (Schewe and Aguirre-Ghiso 2008). ATF6 induces expression of proteins associated with tumor transformation (Arai et al. 2006) and chemo-resistance (Higa et al. 2014), which may explain why recurrent tumors are refractory to second rounds of chemotherapy. Finally, forced expression of the activated form of ATF6p50 caused spontaneous colitis and colon cancer in mice (Coleman et al. 2018).

Nevertheless, due to the mutual compensation of the UPR branches, it is challenging to dissect the exact requirement of each UPR sensor in cancer. For example, inactivation of any of the three UPR pathways may result in compensatory activation of alternative UPR pathways. Therefore, caution is necessary to interpret the causal role of individual UPR signalling events that promote or limit tumor initiation and progression.

8.15 The UPR and Immune Surveillance

The ER plays a pivotal role in the synthesis and assembly of the major histocompatibility complex (MHC). After its polypeptide synthesis and ER membrane translocation, the assembly of the heavy and macroglobulin β2m chains of MHC class I (MHC-I) takes place in the ER lumen. The assembly and the subsequent loading of a high-affinity peptide fragment for immune recognition are facilitated by the TAP channel, tapasin, and by the ER chaperones ERp57, calnexin and calreticulin (Blum et al. 2013). Under the stringent ER protein folding quality control, only appropriately folded MHC-I with a peptide cargo is allowed to be exported to the cell surface for antigen display under immune surveillance. Therefore, ER stress is associated with insufficient MHC-I assembly and cell surface presentation (Granados et al. 2009), which is speculated to cause impaired immune surveillance in the tumor microenvironment. Conversely, the ER chaperone GRP94, an HSP90 family member, is well known for its role in facilitating peptide presentation by professional antigen-presenting cells (APCs) (Schild and Rammensee 2000). This peptide presentation function of GRP94 was further mapped to its N-terminal domain compromising the first 355 amino acids (Biswas et al. 2006). It is plausible that ER stress in tumor cells could interfere with the role of GRP94 in facilitating antigen uptake by APCs for its presentation class I and class II MHC molecules, as misfolded protein cargo in the ER lumen can sequester the HSP-like chaperones (Dorner et al. 1988).

8.16 Therapeutic Strategies to Target the UPR

The complexity of ER stress responses has significant potential for therapeutic intervention. The advantage of targeting the UPR is that tumor cells rely more on this pathway for survival than healthy cells, thus providing strategies for selective killing.

8.17 Chemical Chaperones

Broad spectrum chemical chaperones have been identified as low molecular weight compounds that alleviate ER stress by promoting protein folding, preventing protein aggregation and increasing ERAD. The most common ER stress alleviators are 4-phenylbutyric acid (PBA), a short-chain fatty acid also described as a potent histone deacetylase inhibitor, and tauroursodeoxycholic acid (TUDCA), a hydrophilic taurine conjugate of the bile salt ursodeoxycholic acid (Sarvani et al. 2017). It was first proposed that these chaperones may be potent therapeutic agents against metabolic disorders. Treatment with either PBA or TUDCA efficiently resolved hepatic steatosis and enhanced insulin action in the livers of *ob/ob* mice with type 2 diabetes (Özcan et al. 2006). PBA, and especially TUDCA, were also shown to protect against liver injury and regeneration failure as they reduced inflammation, apoptosis and necrosis in both steatotic and non-steatotic livers after partial hepatectomy and ischemia-reperfusion (Ben Mosbah et al. 2010). In HFD-fed mice and in non-alcoholic ste-

atohepatitis (NASH)-prone transgenic mice that express high levels of misfolded urokinase in hepatocytes, treatment with TUDCA or hepatic overexpression of BiP abrogated the signs of NASH: protecting against hepatic steatosis and liver injury characterized by ballooned hepatocytes, increased ROS and cell death (Nakagawa et al. 2014). Similarly, administration of TUDCA to *ob/ob* leptin-deficient obese mice challenged with lipopolysaccharide protected against ER stress-dependent NLRP3 inflammasome activation and liver injury (Lebeaupin et al. 2015). Oral administration of either PBA or TUDCA in ER stress-prone mice with colitis significantly reduced signs of colonic inflammation by alleviating ER stress in colonic epithelial cells (Cao et al. 2013).

TUDCA and PBA are Food and Drug Administration-approved agents as orally active chemical chaperones that reduce ER stress and are being tested in extensive clinical trials. That being said, these chaperones usually require high concentrations due to their poor selectivity, often making them neglected as therapeutic agents. While PBA and TUDCA have proven their safety and potency in reducing ER stress, their direct mechanisms of action have yet to be clearly defined.

8.18 Pharmacological Inhibitors/Activators

Many pharmacological compounds target distinct UPR signalling molecules (Hetz et al. 2013; Lebeaupin et al. 2018a) (Table 8.1). Nevertheless, many compounds have yet to prove their efficacy and safety *in vivo* to confirm their therapeutic potential.

IRE1α, possessing both a catalytic core in its RNase domain and an ATP-binding pocket in its kinase domain, can be manipulated pharmacologically. The challenge lies in developing selective compounds that interact with one domain without affecting the other. Small molecule inhibitors of the RNase function of IRE1α include 4μ8c (Lebeaupin et al. 2018b; Tufanli et al. 2017), STF-083010 (Kim et al. 2015; Lebeaupin et al. 2018b; Lerner et al. 2012; Tufanli et al. 2017) and MKC-3946 (Mimura et al. 2012). IRE1α RNase function can also be modulated through its kinase domain by ATP-competitive ligands, forming a new pharmacological class of inhibitors called Kinase-Inhibiting RNase Attenuators (KIRAs) (Ghosh et al. 2014; Han et al. 2009; Morita et al. 2017). Through a conformational change, broad-acting kinase inhibitors APY29 (Kuo et al. 2017; Wang et al. 2012) and clinically-approved sunitinib (gastrointestinal, renal and pancreatic cancer) block IRE1α kinase activity, but allosterically activate IRE1α's RNase domain in yeast (Korennykh et al. 2009). Another compound, selonsertib (GS-4997), showed potential in patients with NASH as a well-tolerated selective inhibitor of ASK1, an important intermediary of IRE1α-JNK1 signalling (Loomba et al. 2017), but recently failed a phase 3 clinical trial (Gilead Sciences), further exposing the unmet need for effective liver disease treatments. Selonsertib could nevertheless provide a treatment strategy against multidrug resistance in a variety of cancers (Ji et al. 2019).

Targeting the PERK pathway, the small molecules GSK2606414 (first generation) and GSK2656157 (preclinical development candidate) were developed as pharmacological inhibitors of PERK autophosphorylation (Atkins et al. 2013; Axten et al. 2013), but recent evidence showing off-target effects of these compounds questions their selectivity (Rojas-Rivera et al. 2017). In addition, these compounds destroy pancreatic β-cells, likely because β-cells require PERK-mediated phosphorylation of eIF2α (Mercado et al. 2018). At the expense of pancreatic β-cell survival, GSK2606414 may protect against ER stress-related neurodegenerative decline (Mercado et al. 2018). By preventing eIF2α dephosphorylation through the selective disruption of the PP1-PPP1R15A/GADD34 holoprotein P-eIF2α phosphatase complex, the compounds salubrinal (Vandewynckel et al. 2015), guanabenz (Tsaytler et al. 2011) and its derivative sephin1 (Chen et al. 2019; Das et al. 2015) maintain mRNA translational inhibition. Recent findings challenge the assumption that guanabenz and sephin1 interfere with eIF2α

Table 8.1 Pharmacological compounds that target UPR pathways with therapeutic implications

Name	Target	Mechanism	Potential therapies	References
4μ8c	IRE1α RNase	Inhibits XBP1 mRNA splicing. Inhibits RIDD function.	Atherosclerosis	Tufanli et al. (2017)
			NAFLD	Lebeaupin et al. (2018a, b)
STF-083010			Diabetes	Lerner et al. (2012)
			Inflammatory diseases	Kim et al. (2015)
			Atherosclerosis	Tufanli et al. (2017)
			NAFLD	Lebeaupin et al. (2018a, b)
MKC-3946			Multiple Myeloma	Mimura et al. (2012)
KIRA6/ KIRA8	IRE1α kinase and RNase	Promotes cell survival under ER stress.	Diabetes	Ghosh et al. (2014)
			Diabetes	Morita et al. (2017)
APY29	IRE1α kinase	ATP-competitive inhibitor that inhibits IRE1α kinase, but increases dimerization/oligomerization of IRE1α, enhancing RNase activity.	*ND*	Korennykh et al. (2009)
			ND	Wang et al. (2012)
			Kidney cancer	Kuo et al. (2017)
Selonsertib	ASK1	Inhibits ASK1-JNK1 signaling with potential anti-inflammatory, and anti-fibrotic activities.	NAFLD with fibrosis	Loomba et al. (2017)
			Multidrug resistance	Ji et al. (2019)
GSK2606414 GSK2656157	PERK	Inhibits PERK autophosphorylation.	Cancer (tumor development and angiogenesis)	Axten et al. (2013) Atkins et al. (2013)
			Parkinson's disease	Mercado et al. (2018)
Salubrinal	P-eIF2α	Prevents eIF2α dephosphorylation, maintaining mRNA translation inhibition to limit the ER protein load.	Liver cancer	Vandewynckel et al. (2015)
Guanabenz			*ND*	Tsaytler et al. (2011)
Sephin1			Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis	Das et al. (2015)
			ND	Crespillo-Casado et al. (2017)
			Multiple sclerosis	Chen et al. (2019)
Raphin1			Huntington's disease	Krzyzosiak et al. (2018)
ISRIB 2BAct	P-eIF2α	Inhibits P-eIF2α to resume global mRNA translation.	*ND*	Sidrauski et al. (2015)
			ND	Tsai et al. (2018)
			ND	Zyryanova et al. (2018)
			Vanishing white matter disease	Wong et al. (2019)
ML291	CHOP	Selective CHOP inducer with anti-proliferative effects.	*ND*	Flaherty et al. (2013) and Flaherty et al. (2014)

(continued)

Table 8.1 (continued)

Name	Target	Mechanism	Potential therapies	References
PACMA 31	PDI	PDI inhibitors increases ER stress to reduce cell viability with anti-proliferative effects.	Ovarian cancer	Badolato et al. (2017)
Bacitracin			Glioblastoma	Goplen et al. (2006)
AEBSF	ATF6	Prevents ATF6 proteolytic cleavage in the Golgi and activation.	*ND*	Okada et al. (2003)
Ceapins	ATF6	Traps ATF6 in the ER to prevent activation.	*ND*	Gallagher et al. (2016)
147	ATF6	Localized metabolic activation of ATF6 to enhance proteostasis.	*ND*	Plate et al. (2016)
			ND	Paxman et al. (2018)
			Cardiac ischemia/ reperfusion damage	Blackwood et al. (2019)

ND: therapeutic function in vivo not determined

dephosphorylation (Crespillo-Casado et al. 2017), although both drugs do reduce ER stress. A newly discovered and selective phosphatase inhibitor raphin1 targeting PPP1R15B may have potential as an orally available and selective compound that improves proteostasis in neurodegenerative diseases (Krzyzosiak et al. 2018).

While aiming to prevent eIF2α dephosphorylation may exert protective effects against misfolding and a protein overload in the ER in the short-term, persistent inhibition of global translation in cells is poorly tolerated in the long-term. On the contrary, a potent integrated stress response inhibitor (ISRIB, or the more recent 2BAct) was shown to render cells resistant to the effects of eIF2α phosphorylation via eIF2B activation (Sidrauski et al. 2015; Wong et al. 2019), likely a consequence of stabilizing a dimer of the pentameric eIF2B (Tsai et al. 2018; Zyryanova et al. 2018), permitting continued protein synthesis, protecting UPR function and promoting cell survival. In an effort to selectively activate the apoptotic versus the adaptive arm of the UPR, the chemical probe and sulfonamidebenzamide ML291 was developed (Flaherty et al. 2013). ML291 demonstrated efficacy in inducing CHOP-dependent cell death in a number of cell lines, making it a potential candidate for cancer therapy (Flaherty et al. 2014). PDI antagonists, such as PACMAs (Badolato et al. 2017) and bacitracin (Goplen et al. 2006), also represent important approaches for the development of targeted anticancer compounds.

Agents that specifically modulate ATF6 expression or activity are limited and general serine protease inhibitors, such as AEBSF (Okada et al. 2003), are commonly used. A new class of small molecule inhibitors called Ceapins trap full-length ATF6 in ER-resident foci, preventing ER stress-induced trafficking of ATF6 to the Golgi for proteolytic activation (Gallagher et al. 2016). Another strategy involves reprogramming the ER proteostasis environment through the genetic activation of ATF6α by the small molecule N-(2-hydroxy-5-methylphenyl)-3-phenylpropanamide (147), shown to attenuate secretion and aggregation of amyloidogenic proteins (Paxman et al. 2018; Plate et al. 2016). Treatment of ischemia/reperfused cardiomyocytes with 147 promoted proteostasis and reduced oxidative stress, while 147 administered *in vivo* improved cardiac performance in mice subjected to acute myocardial infarction (Blackwood et al. 2019).

8.19 Antioxidants

Accumulating evidence suggests that protein folding and production of ROS are closely linked, with persistent ER stress and oxidative stress synergistically initiating apoptotic cascades and playing significant roles in disease pathogenesis (Malhotra and Kaufman 2007). The application of antioxi-

dants not only reduces oxidative stress, but also improves protein folding and secretion to prevent ER stress-induced apoptosis (Han et al. 2015; Malhotra et al. 2008). Accumulation of misfolded clotting FVIII in the ER lumen activates the UPR, causes oxidative stress and induces apoptosis. In mice injected with a vector that encodes aggregation-prone FVIII, feeding with butylated hydroxyanisole (BHA), a lipid-soluble antioxidant, reduces levels of UPR activation, oxidative stress and apoptosis and increases FVIII secretion (Malhotra et al. 2008). Mice deficient for *P58*^{IPK}, an ER luminal co-chaperone for BiP, are susceptible to protein-folding defects, reduced pancreatic β-cell mass and function (Han et al. 2015), and multisystemic neuropathy (Synofzik et al. 2014). In these mice the β-cell failure and diabetes were attenuated when fed with a BHA-supplemented diet (Han et al. 2015). Since efforts to reduce ROS are associated with improved protein folding and cell survival, antioxidant treatment may offer a feasible treatment perspective in protein misfolding diseases and metabolic diseases.

In detoxifying organs such as the liver, the antioxidant response is orchestrated by the highly expressed factor NRF2. NRF2 has been suggested to play a cytoprotective role in response to ER stress-dependent inflammation in animal models with NASH (Okada et al. 2012). Further, pharmacological activators of NRF2 signalling significantly reduced fibrosis in rats with diet-induced NASH, demonstrating a potential strategy to treat NASH patients with hepatic fibrosis (Shimozono et al. 2013). However, NRF2 also induces p62 gene expression through a self-amplifying autoregulatory loop that creates an oncogenic environment, protecting hepatocellular carcinoma-initiating cells from oxidative stress-induced death (Jain et al. 2010; Umemura et al. 2016). Thus, antioxidants may promote rather than suppress certain cancer development.

8.20 Conclusion

Since tumor cells frequently exhibit a partially active UPR, there is the possibility to preferentially kill these cells by inducing low levels of ER stress, for which normal cells can tolerate and adapt. There are thus multiple ways to modulate ER physiology in an effort to treat diseases related to abnormal ER stress. To confirm their therapeutic potential, the specificity of different pharmacological compounds should be of particular concern, along with the potential side effects due to the artificial manipulation of the signalling pathways of the UPR, which can transition from adaptive to cell death programs. The application of our understanding of the UPR and downstream signalling will prove instrumental in developing novel therapies for a wide range of diseases.

Acknowledgements This work was supported by the NIH grants R01HL052173, R37DK042394, R24DK110973, R01DK103185, R01DK113171, R01AG062190 and R01CA198103 to R.J.K. and the NCI Cancer Center Support Grant P30 CA030199. R.J.K. is a member of the UCSD DRC (P30 DK063491) and Adjunct Professor in the Department of Pharmacology, UCSD. C.L. is a member of the UCSD DRC and is supported by the NIH grant T32DK007494.

References

Aguirre-Ghiso JA (2007) Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 7:834–846

Amin-Wetzel N, Saunders RA, Kamphuis MJ, Rato C, Preissler S, Harding HP, Ron D (2017) A J-protein co-chaperone recruits BiP to monomerize IRE1 and repress the unfolded protein response. Cell 171:1625–1637.e1613

Arai M, Kondoh N, Imazeki N, Hada A, Hatsuse K, Kimura F, Matsubara O, Mori K, Wakatsuki T, Yamamoto M (2006) Transformation-associated gene regulation by ATF6alpha during hepatocarcinogenesis. FEBS Lett 580:184–190

Atkins C, Liu Q, Minthorn E, Zhang SY, Figueroa DJ, Moss K, Stanley TB, Sanders B, Goetz A, Gaul N et al (2013) Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. Cancer Res 73:1993–2002

Averous J, Bruhat A, Jousse C, Carraro V, Thiel G, Fafournoux P (2004) Induction of CHOP expression by amino acid limitation requires both ATF4 expression and ATF2 phosphorylation. J Biol Chem 279:5288–5297

Axten JM, Romeril SP, Shu A, Ralph J, Medina JR, Feng Y, Li WH, Grant SW, Heerding DA, Minthorn E et al (2013) Discovery of GSK2656157: an optimized

PERK inhibitor selected for preclinical development. ACS Med Chem Lett 4:964–968

Back SH, Scheuner D, Han J, Song B, Ribick M, Wang J, Gildersleeve RD, Pennathur S, Kaufman RJ (2009) Translation attenuation through eIF2alpha phosphorylation prevents oxidative stress and maintains the differentiated state in beta cells. Cell Metab 10:13–26

Badolato M, Carullo G, Aiello F, Garofalo A (2017) Synthesis and experimental validation of new PDI inhibitors with antiproliferative activity. J Chem 2017:1–9

Bailly-Maitre B, Belgardt BF, Jordan SD, Coornaert B, von Freyend MJ, Kleinridders A, Mauer J, Cuddy M, Kress CL, Willmes D et al (2010) Hepatic Bax inhibitor-1 inhibits IRE1alpha and protects from obesity-associated insulin resistance and glucose intolerance. J Biol Chem 285:6198–6207

Ben Mosbah I, Alfany-Fernandez I, Martel C, Zaouali MA, Bintanel-Morcillo M, Rimola A, Rodes J, Brenner C, Rosello-Catafau J, Peralta C (2010) Endoplasmic reticulum stress inhibition protects steatotic and non-steatotic livers in partial hepatectomy under ischemia-reperfusion. Cell Death Dis 1:e52

Biswas C, Sriram U, Ciric B, Ostrovsky O, Gallucci S, Argon Y (2006) The N-terminal fragment of GRP94 is sufficient for peptide presentation via professional antigen-presenting cells. Int Immunol 18:1147–1157

Blackwood EA, Azizi K, Thuerauf DJ, Paxman RJ, Plate L, Kelly JW, Wiseman RL, Glembotski CC (2019) Pharmacologic ATF6 activation confers global protection in widespread disease models by reprograming cellular proteostasis. Nat Commun 10:187

Blais JD, Filipenko V, Bi M, Harding HP, Ron D, Koumenis C, Wouters BG, Bell JC (2004) Activating transcription factor 4 is translationally regulated by hypoxic stress. Mol Cell Biol 24:7469–7482

Blum JS, Wearsch PA, Cresswell P (2013) Pathways of antigen processing. Annu Rev Immunol 31:443–473

Cao SS, Kaufman RJ (2014) Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxid Redox Signal 21:396–413

Cao SS, Zimmermann EM, Chuang BM, Song B, Nwokoye A, Wilkinson JE, Eaton KA, Kaufman RJ (2013) The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. Gastroenterology 144:989–1000.e6

Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS et al (2007) The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell 11:349–360

Carreras-Sureda A, Jana F, Urra H, Durand S, Mortenson DE, Sagredo A, Bustos G, Hazari Y, Ramos-Fernandez E, Sassano ML et al (2019) Non-canonical function of IRE1alpha determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. Nat Cell Biol 21:755–767

Cerezo M, Lehraiki A, Millet A, Rouaud F, Plaisant M, Jaune E, Botton T, Ronco C, Abbe P, Amdouni H et al (2016) Compounds triggering ER stress exert anti-melanoma effects and overcome BRAF inhibitor resistance. Cancer Cell 29:805–819

Chen X, Iliopoulos D, Zhang Q, Tang Q, Greenblatt MB, Hatziapostolou M, Lim E, Tam WL, Ni M, Chen Y et al (2014) XBP1 promotes triple-negative breast cancer by controlling the HIF1alpha pathway. Nature 508:103–107

Chen Y, Podojil JR, Kunjamma RB, Jones J, Weiner M, Lin W, Miller SD, Popko B (2019) Sephin1, which prolongs the integrated stress response, is a promising therapeutic for multiple sclerosis. Brain 142:344–361

Coleman OI, Lobner EM, Bierwirth S, Sorbie A, Waldschmitt N, Rath E, Berger E, Lagkouvardos I, Clavel T, McCoy KD et al (2018) Activated ATF6 induces intestinal dysbiosis and innate immune response to promote colorectal tumorigenesis. Gastroenterology 155:1539–1552.e12

Crespillo-Casado A, Chambers JE, Fischer PM, Marciniak SJ, Ron D (2017) PPP1R15A-mediated dephosphorylation of eIF2alpha is unaffected by Sephin1 or Guanabenz. Elife 6:pii: e26109

Cubillos-Ruiz JR, Bettigole SE, Glimcher LH (2017) Tumorigenic and immunosuppressive effects of endoplasmic reticulum stress in cancer. Cell 168:692–706

Cullinan SB, Diehl JA (2004) PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. J Biol Chem 279:20108–20117

Cullinan SB, Zhang D, Hannink M, Arvisais E, Kaufman RJ, Diehl JA (2003) Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. Mol Cell Biol 23:7198–7209

Das I, Krzyzosiak A, Schneider K, Wrabetz L, D'Antonio M, Barry N, Sigurdardottir A, Bertolotti A (2015) Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. Science 348:239

Dejeans N, Pluquet O, Lhomond S, Grise F, Bouchecareilh M, Juin A, Meynard-Cadars M, Bidaud-Meynard A, Gentil C, Moreau V et al (2012) Autocrine control of glioma cells adhesion and migration through IRE1α-mediated cleavage of SPARC mRNA. J Cell Sci 125:4278

Delepine M, Nicolino M, Barrett T, Golamaully M, Lathrop GM, Julier C (2000) EIF2AK3, encoding translation initiation factor 2-alpha kinase 3, is mutated in patients with Wolcott-Rallison syndrome. Nat Genet 25:406–409

Denoyelle C, Abou-Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, Pointer JN, Gruber SB, Su LD, Nikiforov MA et al (2006) Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. Nat Cell Biol 8:1053–1063

Dong D, Stapleton C, Luo B, Xiong S, Ye W, Zhang Y, Jhaveri N, Zhu G, Ye R, Liu Z et al (2011) A critical role for GRP78/BiP in the tumor microenvironment for neovascularization during tumor growth and metastasis. Cancer Res 71:2848–2857

Dorner AJ, Krane MG, Kaufman RJ (1988) Reduction of endogenous GRP78 levels improves secretion of

a heterologous protein in CHO cells. Mol Cell Biol 8:4063–4070

Dorner AJ, Wasley LC, Kaufman RJ (1990) Protein dissociation from GRP78 and secretion are blocked by depletion of cellular ATP levels. Proc Natl Acad Sci U S A 87:7429–7432

Dorner AJ, Wasley LC, Kaufman RJ (1992) Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. EMBO J 11:1563–1571

Eletto D, Eletto D, Dersh D, Gidalevitz T, Argon Y (2014) Protein disulfide isomerase A6 controls the decay of IRE1alpha signaling via disulfide-dependent association. Mol Cell 53:562–576

Feng YX, Sokol ES, Del Vecchio CA, Sanduja S, Claessen JH, Proia TA, Jin DX, Reinhardt F, Ploegh HL, Wang Q et al (2014) Epithelial-to-mesenchymal transition activates PERK-eIF2alpha and sensitizes cells to endoplasmic reticulum stress. Cancer Discov 4:702–715

Flaherty D, Golden J, Liu C, Hedrick M, Gosalia P, Liou Y, Milewski M, Sugarman E, Suyama E, Nguyen K et al (2013) Selective small molecule activator of the apoptotic arm of the UPR. In (Bethesda (MD): National Center for Biotechnology Information: Probe Reports from the NIH Molecular Libraries Program)

Flaherty DP, Miller JR, Garshott DM, Hedrick M, Gosalia P, Li Y, Milewski M, Sugarman E, Vasile S, Salaniwal S et al (2014) Discovery of sulfonamidebenzamides as selective apoptotic CHOP pathway activators of the unfolded protein response. ACS Med Chem Lett 5:1278–1283

Fu Y, Li J, Lee AS (2007) GRP78/BiP inhibits endoplasmic reticulum BIK and protects human breast cancer cells against estrogen starvation-induced apoptosis. Cancer Res 67:3734–3740

Gallagher CM, Garri C, Cain EL, Ang KK, Wilson CG, Chen S, Hearn BR, Jaishankar P, Aranda-Diaz A, Arkin MR et al (2016) Ceapins are a new class of unfolded protein response inhibitors, selectively targeting the ATF6alpha branch. Elife 5:pii: e11878

Ghosh R, Wang L, Wang ES, Perera BG, Igbaria A, Morita S, Prado K, Thamsen M, Caswell D, Macias H et al (2014) Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress. Cell 158:534–548

Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volker SE, Pambuccian SE, Ondrey FG, Adams GL, Gaffney PM (2004) Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. Cancer Res 64:55–63

Goplen D, Wang J, Enger PO, Tysnes BB, Terzis AJ, Laerum OD, Bjerkvig R (2006) Protein disulfide isomerase expression is related to the invasive properties of malignant glioma. Cancer Res 66:9895–9902

Granados DP, Tanguay PL, Hardy MP, Caron E, de Verteuil D, Meloche S, Perreault C (2009) ER stress affects processing of MHC class I-associated peptides. BMC Immunol 10:10

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C et al (2007) Patterns of somatic mutation in human cancer genomes. Nature 446:153–158

Groenendyk J, Peng Z, Dudek E, Fan X, Mizianty MJ, Dufey E, Urra H, Sepulveda D, Rojas-Rivera D, Lim Y et al (2014) Interplay between the oxidoreductase PDIA6 and microRNA-322 controls the response to disrupted endoplasmic reticulum calcium homeostasis. Sci Signal 7:ra54

Gupta A, Hossain MM, Miller N, Kerin M, Callagy G, Gupta S (2016) NCOA3 coactivator is a transcriptional target of XBP1 and regulates PERK–eIF2α–ATF4 signalling in breast cancer. Oncogene 35:5860

Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, Papa FR (2009) IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 138:562–575

Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, Yuan CL, Krokowski D, Wang S, Hatzoglou M et al (2013) ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat Cell Biol 15:481–490

Han J, Song B, Kim J, Kodali VK, Pottekat A, Wang M, Hassler J, Wang S, Pennathur S, Back SH et al (2015) Antioxidants complement the requirement for protein chaperone function to maintain β-cell function and glucose homeostasis. Diabetes 64:2892–2904

Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R et al (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 11:619–633

Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, Qiu B, Zhang H, Cerniglia G, Bi M et al (2012) ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest 122:4621–4634

Hassler JR, Scheuner DL, Wang S, Han J, Kodali VK, Li P, Nguyen J, George JS, Davis C, Wu SP et al (2015) The IRE1alpha/XBP1s pathway is essential for the glucose response and protection of beta cells. PLoS Biol 13:e1002277

Haze K, Yoshida H, Yanagi H, Yura T, Mori K (1999) Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 10:3787–3799

Hetz C, Glimcher LH (2009) Fine-tuning of the unfolded protein response: assembling the IRE1α interactome. Mol Cell 35:551–561

Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glimcher LH et al (2006) Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. Science 312:572–576

Hetz C, Martinon F, Rodriguez D, Glimcher LH (2011) The unfolded protein response: integrating stress signals through the stress sensor IRE1alpha. Physiol Rev 91:1219–1243

Hetz C, Chevet E, Harding HP (2013) Targeting the unfolded protein response in disease. Nat Rev Drug Discov 12:703–719

Hetz C, Chevet E, Oakes SA (2015) Proteostasis control by the unfolded protein response. Nat Cell Biol 17:829–838

Higa A, Taouji S, Lhomond S, Jensen D, Fernandez-Zapico ME, Simpson JC, Pasquet JM, Schekman R, Chevet E (2014) Endoplasmic reticulum stress-activated transcription factor ATF6alpha requires the disulfide isomerase PDIA5 to modulate chemoresistance. Mol Cell Biol 34:1839–1849

Hollien J, Weissman JS (2006) Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. Science 313:104

Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS (2009) Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol 186:323

Hong SY, Hagen T (2013) Multiple myeloma Leu167Ile (c.499C>A) mutation prevents XBP1 mRNA splicing. Br J Haematol 161:898–901

Huber AL, Lebeau J, Guillaumot P, Petrilli V, Malek M, Chilloux J, Fauvet F, Payen L, Kfoury A, Renno T et al (2013) p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression upon low glucose. Mol Cell 49:1049–1059

Jain A, Lamark T, Sjottem E, Larsen KB, Awuh JA, Overvatn A, McMahon M, Hayes JD, Johansen T (2010) p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. J Biol Chem 285:22576–22591

Ji N, Yang Y, Cai CY, Lei ZN, Wang JQ, Gupta P, Shukla S, Ambudkar SV, Kong D, Chen ZS (2019) Selonsertib (GS-4997), an ASK1 inhibitor, antagonizes multidrug resistance in ABCB1- and ABCG2-overexpressing cancer cells. Cancer Lett 440–441:82–93

Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, Yue P, Haverty PM, Bourgon R, Zheng J et al (2010) Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 466:869–873

Karagoz GE, Acosta-Alvear D, Nguyen HT, Lee CP, Chu F, Walter P (2017) An unfolded protein-induced conformational switch activates mammalian IRE1. Elife 6:pii: e30700

Kaufman RJ (2004) Regulation of mRNA translation by protein folding in the endoplasmic reticulum. Trends Biochem Sci 29:152–158

Kaufman RJ, Malhotra JD (2014) Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics. Biochim Biophys Acta 1843:2233–2239

Kim S, Joe Y, Kim HJ, Kim YS, Jeong SO, Pae HO, Ryter SW, Surh YJ, Chung HT (2015) Endoplasmic reticulum stress-induced IRE1alpha activation mediates cross-talk of GSK-3beta and XBP-1 to regulate inflammatory cytokine production. J Immunol 194:4498–4506

Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P (2009) The unfolded protein response signals through high-order assembly of Ire1. Nature 457:687–693

Krzyzosiak A, Sigurdardottir A, Luh L, Carrara M, Das I, Schneider K, Bertolotti A (2018) Target-based discovery of an inhibitor of the regulatory phosphatase PPP1R15B. Cell 174:1216–1228.e19

Kuo CY, Lin CH, Hsu T (2017) VHL inactivation in precancerous kidney cells induces an inflammatory response via ER stress-activated IRE1alpha signaling. Cancer Res 77:3406–3416

Kwong JQ (2017) The mitochondrial calcium uniporter in the heart: energetics and beyond. J Physiol 595:3743–3751

Kwong JQ, Lu X, Correll RN, Schwanekamp JA, Vagnozzi RJ, Sargent MA, York AJ, Zhang J, Bers DM, Molkentin JD (2015) The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. Cell Rep 12:15–22

La Rovere RM, Roest G, Bultynck G, Parys JB (2016) Intracellular Ca(2+) signaling and Ca(2+) microdomains in the control of cell survival, apoptosis and autophagy. Cell Calcium 60:74–87

Lebeaupin C, Proics E, de Bieville CH, Rousseau D, Bonnafous S, Patouraux S, Adam G, Lavallard VJ, Rovere C, Le Thuc O et al (2015) ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis 6:e1879

Lebeaupin C, Vallee D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B (2018a) Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. J Hepatol 69:927–947

Lebeaupin C, Vallee D, Rousseau D, Patouraux S, Bonnafous S, Adam G, Luciano F, Luci C, Anty R, Iannelli A et al (2018b) Bax inhibitor-1 protects from nonalcoholic steatohepatitis by limiting inositol-requiring enzyme 1 alpha signaling in mice. Hepatology 68:515–532

Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F (2008) Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing. Cell 132:89–100

Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M et al (2012) IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. Cell Metab 16:250–264

Leung-Hagesteijn C, Erdmann N, Cheung G, Keats JJ, Stewart AK, Reece DE, Chung KC, Tiedemann RE (2013) Xbp1s-negative tumor B cells and preplasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. Cancer Cell 24:289–304

Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultsinos D, McMahon M, Pineau R, Obacz J, Papadodima

O, Jouan F et al (2018) Dual IRE1 RNase functions dictate glioblastoma development. EMBO Mol Med 10(3):pii: e7929

Li G, Mongillo M, Chin KT, Harding H, Ron D, Marks AR, Tabas I (2009) Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. J Cell Biol 186:783–792

Lin YH, Friederichs J, Black MA, Mages J, Rosenberg R, Guilford PJ, Phillips V, Thompson-Fawcett M, Kasabov N, Toro T et al (2007) Multiple gene expression classifiers from different array platforms predict poor prognosis of colorectal cancer. Clin Cancer Res 13:498–507

Lisbona F, Rojas-Rivera D, Thielen P, Zamorano S, Todd D, Martinon F, Glavic A, Kress C, Lin JH, Walter P et al (2009) BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1alpha. Mol Cell 33:679–691

Liu JC, Liu J, Holmstrom KM, Menazza S, Parks RJ, Fergusson MM, Yu ZX, Springer DA, Halsey C, Liu C et al (2016) MICU1 serves as a molecular gatekeeper to prevent in vivo mitochondrial calcium overload. Cell Rep 16:1561–1573

Loomba R, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, Diehl AM, Djedjos CS, Han L, Myers RP et al (2017) The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. Hepatology 67(2):549–559

Luciani DS, Gwiazda KS, Yang TLB, Kalynyak TB, Bychkivska Y, Frey MHZ, Jeffrey KD, Sampaio AV, Underhill TM, Johnson JD (2008) Roles of IP3R and RyR Ca2+ channels in endoplasmic reticulum stress and beta-cell death. Diabetes 58:422–432

Ma Y, Hendershot LM (2003) Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. J Biol Chem 278:34864–34873

Ma X-H, Piao S-F, Dey S, McAfee Q, Karakousis G, Villanueva J, Hart LS, Levi S, Hu J, Zhang G et al (2014) Targeting ER stress–induced autophagy overcomes BRAF inhibitor resistance in melanoma. J Clin Invest 124:1406–1417

Malhotra JD, Kaufman RJ (2007) Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid Redox Signal 9:2277–2293

Malhotra JD, Miao H, Zhang K, Wolfson A, Pennathur S, Pipe SW, Kaufman RJ (2008) Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. Proc Natl Acad Sci U S A 105:18525–18530

Mallilankaraman K, Cardenas C, Doonan PJ, Chandramoorthy HC, Irrinki KM, Golenar T, Csordas G, Madireddi P, Yang J, Muller M et al (2012) MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism. Nat Cell Biol 14:1336–1343

Marciniak SJ, Ron D (2006) Endoplasmic reticulum stress signaling in disease. Physiol Rev 86:1133–1149

Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D (2004) CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev 18:3066–3077

Maurel M, Chevet E, Tavernier J, Gerlo S (2014) Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci 39:245–254

Mercado G, Castillo V, Soto P, Lopez N, Axten JM, Sardi SP, Hoozemans JJM, Hetz C (2018) Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. Neurobiol Dis 112:136–148

Miao HZ, Sirachainan N, Palmer L, Kucab P, Cunningham MA, Kaufman RJ, Pipe SW (2004) Bioengineering of coagulation factor VIII for improved secretion. Blood 103:3412–3419

Milani M, Rzymski T, Mellor HR, Pike L, Bottini A, Generali D, Harris AL (2009) The role of ATF4 stabilization and autophagy in resistance of breast cancer cells treated with Bortezomib. Cancer Res 69:4415–4423

Mimura N, Fulciniti M, Gorgun G, Tai YT, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H et al (2012) Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. Blood 119:5772–5781

Mimura J, Inose-Maruyama A, Taniuchi S, Kosaka K, Yoshida H, Yamazaki H, Kasai S, Harada N, Kaufman RJ, Oyadomari S et al (2019) Concomitant Nrf2- and ATF4-activation by carnosic acid cooperatively induces expression of cytoprotective genes. Int J Mol Sci 20:pii: E1706

Morita S, Villalta SA, Feldman HC, Register AC, Rosenthal W, Hoffmann-Petersen IT, Mehdizadeh M, Ghosh R, Wang L, Colon-Negron K et al (2017) Targeting ABL-IRE1alpha signaling spares ER-stressed pancreatic beta cells to reverse autoimmune diabetes. Cell Metab 25:883–897.e8

Mujcic H, Nagelkerke A, Rouschop KM, Chung S, Chaudary N, Span PN, Clarke B, Milosevic M, Sykes J, Hill RP et al (2013) Hypoxic activation of the PERK/eIF2alpha arm of the unfolded protein response promotes metastasis through induction of LAMP3. Clin Cancer Res 19:6126–6137

Nadanaka S, Yoshida H, Kano F, Murata M, Mori K (2004) Activation of mammalian unfolded protein response is compatible with the quality control system operating in the endoplasmic reticulum. Mol Biol Cell 15:2537–2548

Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, Zhong Z, Valasek MA, Seki E, Hidalgo J et al (2014) ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. Cancer Cell 26:331–343

Niederreiter L, Fritz TM, Adolph TE, Krismer AM, Offner FA, Tschurtschenthaler M, Flak MB, Hosomi S, Tomczak MF, Kaneider NC et al (2013) ER stress transcription factor Xbp1 suppresses intestinal tumorigenesis and directs intestinal stem cells. J Exp Med 210:2041–2056

Novoa I, Zeng H, Harding HP, Ron D (2001) Feedback inhibition of the unfolded protein response by

GADD34-mediated dephosphorylation of eIF2α. J Cell Biol 153:1011

Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K et al (2006) Autophagy is activated for cell survival after endoplasmic reticulum stress. Mol Cell Biol 26:9220

Okada T, Haze K, Nadanaka S, Yoshida H, Seidah NG, Hirano Y, Sato R, Negishi M, Mori K (2003) A serine protease inhibitor prevents endoplasmic reticulum stress-induced cleavage but not transport of the membrane-bound transcription factor ATF6. J Biol Chem 278:31024–31032

Okada K, Warabi E, Sugimoto H, Horie M, Tokushige K, Ueda T, Harada N, Taguchi K, Hashimoto E, Itoh K et al (2012) Nrf2 inhibits hepatic iron accumulation and counteracts oxidative stress-induced liver injury in nutritional steatohepatitis. J Gastroenterol 47:924–935

Oyadomari S (2002) Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. J Clin Investig 109:525–532

Özcan U, Yilmaz E, Özcan L, Furuhashi M, Vaillancourt E, Smith RO, Görgün CZ, Hotamisligil GS (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313:1137–1140

Paez D, Labonte MJ, Bohanes P, Zhang W, Benhanim L, Ning Y, Wakatsuki T, Loupakis F, Lenz HJ (2012) Cancer dormancy: a model of early dissemination and late cancer recurrence. Clin Cancer Res 18:645–653

Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA et al (2013) The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. Nat Cell Biol 15:1464–1472

Paxman R, Plate L, Blackwood EA, Glembotski C, Powers ET, Wiseman RL, Kelly JW (2018) Pharmacologic ATF6 activating compounds are metabolically activated to selectively modify endoplasmic reticulum proteins. Elife 7:pii: e37168

Pipe SW, Saenko EL, Eickhorst AN, Kemball-Cook G, Kaufman RJ (2001) Hemophilia A mutations associated with 1-stage/2-stage activity discrepancy disrupt protein-protein interactions within the triplicated A domains of thrombin-activated factor VIIIa. Blood 97:685

Plate L, Cooley CB, Chen JJ, Paxman RJ, Gallagher CM, Madoux F, Genereux JC, Dobbs W, Garza D, Spicer TP et al (2016) Small molecule proteostasis regulators that reprogram the ER to reduce extracellular protein aggregation. Elife 5:pii: e15550

Pluquet O, Dejeans N, Bouchecareilh M, Lhomond S, Pineau R, Higa A, Delugin M, Combe C, Loriot S, Cubel G et al (2013) Posttranscriptional regulation of PER1 underlies the oncogenic function of IREα. Cancer Res 73:4732

Pyrko P, Schonthal AH, Hofman FM, Chen TC, Lee AS (2007) The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. Cancer Res 67:9809–9816

Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang CH, Angelo M, Ladd C, Reich M, Latulippe E, Mesirov JP et al (2001) Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci U S A 98:15149–15154

Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS (2003) Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem *278*:20915–20924

Rojas-Rivera D, Delvaeye T, Roelandt R, Nerinckx W, Augustyns K, Vandenabeele P, Bertrand MJM (2017) When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. Cell Death Differ 24:1100–1110

Rouschop KM, Dubois LJ, Keulers TG, van den Beucken T, Lambin P, Bussink J, van der Kogel AJ, Koritzinsky M, Wouters BG (2013) PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. Proc Natl Acad Sci U S A 110:4622–4627

Rzymski T, Milani M, Singleton DC, Harris AL (2009) Role of ATF4 in regulation of autophagy and resistance to drugs and hypoxia. Cell Cycle 8:3838–3847

Sancak Y, Markhard AL, Kitami T, Kovacs-Bogdan E, Kamer KJ, Udeshi ND, Carr SA, Chaudhuri D, Clapham DE, Li AA et al (2013) EMRE is an essential component of the mitochondrial calcium uniporter complex. Science 342:1379–1382

Sarvani C, Sireesh D, Ramkumar KM (2017) Unraveling the role of ER stress inhibitors in the context of metabolic diseases. Pharmacol Res 119:412–421

Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, Saunders T, Bonner-Weir S, Kaufman RJ (2001) Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell 7:1165–1176

Scheuner D, Vander Mierde D, Song B, Flamez D, Creemers JW, Tsukamoto K, Ribick M, Schuit FC, Kaufman RJ (2005) Control of mRNA translation preserves endoplasmic reticulum function in beta cells and maintains glucose homeostasis. Nat Med 11:757–764

Schewe DM, Aguirre-Ghiso JA (2008) ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. Proc Natl Acad Sci U S A 105:10519–10524

Schild H, Rammensee HG (2000) gp96 – the immune system's Swiss army knife. Nat Immunol 1:100–101

Sepulveda D, Rojas-Rivera D, Rodriguez DA, Groenendyk J, Kohler A, Lebeaupin C, Ito S, Urra H, Carreras-Sureda A, Hazari Y et al (2018) Interactome screening identifies the ER luminal chaperone Hsp47 as a regulator of the unfolded protein response transducer IRE1alpha. Mol Cell 69:238–252.e7

Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, Vrbanac AF, Li W, Perkins A, Matsutani T et al (2017) Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. Nature 551:340–345

Shen J, Chen X, Hendershot L, Prywes R (2002) ER Stress Regulation of ATF6 Localization by Dissociation of BiP/GRP78 Binding and Unmasking of Golgi Localization Signals. Dev Cell 3:99–111

Shimozono R, Asaoka Y, Yoshizawa Y, Aoki T, Noda H, Yamada M, Kaino M, Mochizuki H (2013) Nrf2 activators attenuate the progression of nonalcoholic steatohepatitis-related fibrosis in a dietary rat model. Mol Pharmacol 84:62–70

Sidrauski C, McGeachy AM, Ingolia NT, Walter P (2015) The small molecule ISRIB reverses the effects of eIF2alpha phosphorylation on translation and stress granule assembly. Elife 4:e05033

Song B, Scheuner D, Ron D, Pennathur S, Kaufman RJ (2008) Chop deletion reduces oxidative stress, improves β cell function, and promotes cell survival in multiple mouse models of diabetes. J Clin Invest 118:3378–3389

Southwood CM, Fykkolodziej B, Maheras KJ, Garshott DM, Estill M, Fribley AM, Gow A (2016) Overexpression of CHOP in myelinating cells does not confer a significant phenotype under normal or metabolic stress conditions. J Neurosci 36:6803–6819

Sun H, Lin DC, Guo X, Kharabi Masouleh B, Gery S, Cao Q, Alkan S, Ikezoe T, Akiba C, Paquette R et al (2016) Inhibition of IRE1alpha-driven pro-survival pathways is a promising therapeutic application in acute myeloid leukemia. Oncotarget 7:18736–18749

Synofzik M, Haack TB, Kopajtich R, Gorza M, Rapaport D, Greiner M, Schonfeld C, Freiberg C, Schorr S, Holl RW et al (2014) Absence of BiP co-chaperone DNAJC3 causes diabetes mellitus and multisystemic neurodegeneration. Am J Hum Genet 95:689–697

Tsai JC, Miller-Vedam LE, Anand AA, Jaishankar P, Nguyen HC, Renslo AR, Frost A, Walter P (2018) Structure of the nucleotide exchange factor eIF2B reveals mechanism of memory-enhancing molecule. Science 359:pii: eaaq0939

Tsaytler P, Harding HP, Ron D, Bertolotti A (2011) Selective Inhibition of a Regulatory Subunit of Protein Phosphatase 1 Restores Proteostasis. Science 332:91

Tu BP, Weissman JS (2004) Oxidative protein folding in eukaryotes: mechanisms and consequences. J Cell Biol 164:341–346

Tufanli O, Telkoparan Akillilar P, Acosta-Alvear D, Kocaturk B, Onat UI, Hamid SM, Cimen I, Walter P, Weber C, Erbay E (2017) Targeting IRE1 with small molecules counteracts progression of atherosclerosis. Proc Natl Acad Sci U S A 114:E1395–E1404

Umemura A, He F, Taniguchi K, Nakagawa H, Yamachika S, Font-Burgada J, Zhong Z, Subramaniam S, Raghunandan S, Duran A et al (2016) p62, upregulated during preneoplasia, induces hepatocellular carcinogenesis by maintaining survival of stressed HCC-initiating cells. Cancer Cell 29:935–948

Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287:664

Vandewynckel Y-P, Laukens D, Bogaerts E, Paridaens A, Van den Bussche A, Verhelst X, Van Steenkiste C, Descamps B, Vanhove C, Libbrecht L et al (2015) Modulation of the unfolded protein response impedes tumor cell adaptation to proteotoxic stress: a PERK for hepatocellular carcinoma therapy. Hepatol Int 9:93–104

Waldeck-Weiermair M, Malli R, Parichatikanond W, Gottschalk B, Madreiter-Sokolowski CT, Klec C, Rost R, Graier WF (2015) Rearrangement of MICU1 multimers for activation of MCU is solely controlled by cytosolic Ca(2.). Sci Rep 5:15602

Wang M, Kaufman RJ (2014) The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 14:581–597

Wang M, Kaufman RJ (2016) Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 529:326–335

Wang XZ, Kuroda M, Sok J, Batchvarova N, Kimmel R, Chung P, Zinszner H, Ron D (1998) Identification of novel stress-induced genes downstream of chop. EMBO J 17:3619–3630

Wang L, Perera BG, Hari SB, Bhhatarai B, Backes BJ, Seeliger MA, Schurer SC, Oakes SA, Papa FR, Maly DJ (2012) Divergent allosteric control of the IRE1alpha endoribonuclease using kinase inhibitors. Nat Chem Biol 8:982–989

Wong YL, LeBon L, Basso AM, Kohlhaas KL, Nikkel AL, Robb HM, Donnelly-Roberts DL, Prakash J, Swensen AM, Rubinstein ND et al (2019) eIF2B activator prevents neurological defects caused by a chronic integrated stress response. Elife 8:pii: e42940

Wu J, Rutkowski DT, Dubois M, Swathirajan J, Saunders T, Wang J, Song B, Yau GD, Kaufman RJ (2007) ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell 13:351–364

Yong J, Itkin-Ansari P, Kaufman RJ (2016) When less is better: ER stress and beta cell proliferation. Dev Cell 36:4–6

Yong J, Bischof H, Siirin M, Murphy A, Malli R, Kaufman RJ (2019) Mitochondria supply ATP to the ER through a mechanism antagonized by cytosolic Ca^{2+}. Elife 8:pii: e49682

Zyryanova AF, Weis F, Faille A, Alard AA, Crespillo-Casado A, Sekine Y, Harding HP, Allen F, Parts L, Fromont C et al (2018) Binding of ISRIB reveals a regulatory site in the nucleotide exchange factor eIF2B. Science 359:1533

Part III

Therapies

9 The Right Tool for the Job: An Overview of Hsp90 Inhibitors

John Koren III and Brian S. J. Blagg

Abstract

Molecular chaperones are responsible for maintaining intracellular protein quality control by facilitating the conformational maturation of new proteins as well as the refolding of denatured proteins. While there are several classes of molecular chaperones in the cell, this chapter will focus solely on the small molecule modulation of Hsp90, the 90 kDa heat shock protein. Hsp90 is not only responsible for folding nascent proteins, but it also regulates the triage of numerous client proteins through partnering with the ubiquitin-proteasome pathway. Consequently, Hsp90 plays critical role in maintaining the protein homeostasis (proteostasis) network within the cell and is required for the activation/maturation of more than 300 client protein substrates. Many of the clients that depend upon Hsp90 are overexpressed or mutated during malignant transformation. This often renders the clients thermodynamically unstable and dependent on Hsp90 for stability. This phenomenon results in an oncogenic 'addiction' to the Hsp90 protein folding machinery as Hsp90 maintains onco-client proteins. Furthermore, Hsp90-dependent substrates are associated with all ten hallmarks of cancer, making Hsp90 an attractive target for the development of cancer chemotherapeutics. In fact, 17 small molecule inhibitors of Hsp90 have been developed and clinically evaluated for the treatment of cancer. Unfortunately, most of these molecules have failed for various reasons, necessitating a new approach to modulate the Hsp90 protein folding machine.

Keywords

HSP90 inhibitor · Chaperones · Cancer · Heat shock response · Proteostasis · Translational research · Small molecules · Client proteins

9.1 Introduction

There are at least three distinct mechanisms to modulate Hsp90 with small molecules (Fig. 9.1). The N-terminal domain contains an atypical nucleotide-binding site that is responsible for the hydrolysis of ATP, which serves as the requisite source of energy during the protein folding process. In fact, all four Hsp90 isoforms exhibit >85% identity within this region. Due to the high identity among the ATP-binding sites, the Hsp90 inhibitors

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_9

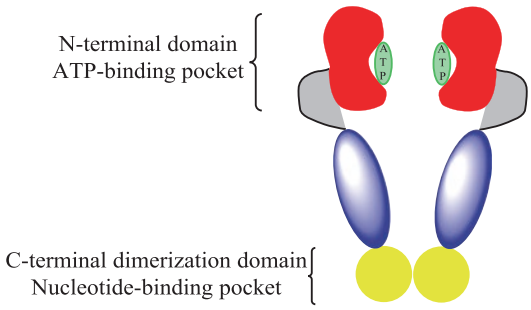


Fig. 9.1 The Hsp90 dimer and points for small molecule modulation

that have undergone clinical evaluation exhibit similar affinity for all four isoforms and manifest pan-inhibitory activity. Upon inhibition of the N-terminal nucleotide binding site, client proteins are unable to achieve their native conformation and instead, become ubiquitinylated and then degraded via the proteasome. At the same concentration of inhibitor needed to induce the degradation of Hsp90-dependent client proteins, the heat shock response (HSR) also occurs. The HSR is a pro-survival response to cellular stress and the accumulation of misfolded proteins, which results in the transcriptional activation of multiple chaperone networks, including Hsp27, Hsp40, Hsp70, and also Hsp90. Thus, inhibitors of the N-terminal domain of Hsp90 not only induce the degradation of oncogenic proteins, but they also induce the levels of Hsp90, which is contraindicated.

Unlike the N-terminal nucleotide binding site, which has been highly sought after for therapeutic development, the C-terminal nucleotide binding site has been less pursued and at present, no co-crystal structure of inhibitors bound to this domain exist. However, multiple studies have now demonstrated that there are several mechanisms to control the Hsp90 protein folding process through modulation of the C-terminal region. In fact, it has been shown that induction of the heat shock response can be segregated from client protein degradation through inhibition of this binding pocket. Thus, compounds targeting this region offer the potential to overcome some of the detriments associated with N-terminal inhibition for the treatment of cancer, in particular, induction of Hsp90 levels. In contrast to the compounds that induce client protein degradation without concomitant induction of the heat shock response, small molecules have also been developed that induce the heat shock response without client protein degradation. Thus, two classes of Hsp90 C-terminal inhibitors have been developed that manifest opposing properties, those that exhibit anti-cancer activity and those that are pro-survival and neuroprotective.

In addition to the N- and C-terminal nucleotide binding sites, natural products have been identified that inhibit the Hsp90-mediated protein folding machinery through disruption of the interactions between co-chaperones and Hsp90, which are required for the maturation of most Hsp90-dependent substrates. Several natural products have been shown to disrupt these protein-protein interactions, and ultimately manifest in distinct inhibitory activities. While none of these molecules are currently undergoing clinical evaluation, they do exhibit properties distinct from N- and C-terminal inhibitors that may be clinically useful. Studies directed toward the development of such inhibitors will be briefly described in this chapter.

9.2 Hsp90 N-Terminal Inhibitors

The N-terminus of Hsp90 contains an ATP-binding pocket. This site, where ATP binds and is hydrolyzed to ADP during the Hsp90-folding process, is the primary location for the binding of numerous Hsp90 inhibitory classes. Kamal et al. were the first to describe an increase in the affinity of Hsp90 for ATP, as well as for ATP-competitive inhibitors, in cancer cells (as compared to normal non-transformed cells) (Kamal et al. 2003). A similar phenomenon has also been described by Dickey et al. following the examination of Hsp90 from Alzheimer's diseased brain (Dickey et al. 2007). Concurrent studies, including the original work by Kamal et al., noted that Hsp90 with altered ATP affinity interacted with a distinct repertoire of co-chaperones (Kamal et al. 2003; Dickey et al. 2007; Moulick et al. 2011; Rodina et al. 2016). In fact, the Chiosis group has published extensively

on this topic, and more recently described an extensive characterization of these complexes in cancer cells, endowing the name 'epichaperome' for these disease-specific Hsp90 complexes (Rodina et al. 2016). These phenomena, although still poorly understood, have served as the impetus for the clinical application of multiple N-terminal Hsp90 inhibitors for potential treatment of cancer and Alzheimer's disease. Ongoing research with existing and novel N-terminal Hsp90 inhibitors has also served to generate valuable chemical tools to investigate Hsp90 function, the disease proteome, as well as probes for detecting and monitoring disease pathogenesis. Herein, we will describe how N-terminal Hsp90 inhibitors have enhanced our understanding of Hsp90 biology in the presence and absence of disease stress.

Geldanamycin (Fig. 9.2), a benzoquinone ansamycin (DeBoer et al. 1970), was the first identified Hsp90 inhibitor, and was initially investigated for its anti-tumor properties. Initially, it was

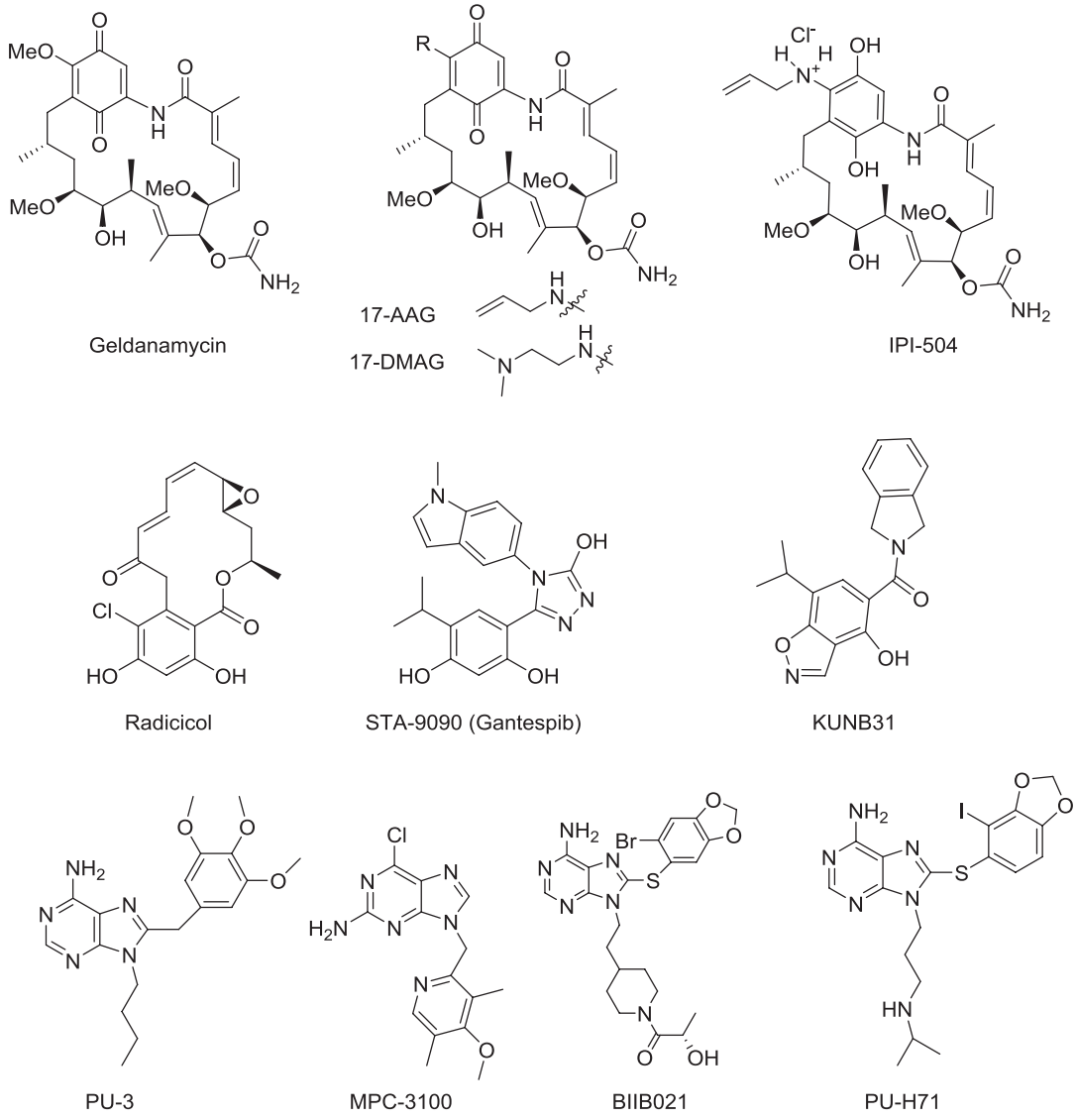


Fig. 9.2 N-terminal Hsp90 inhibitors

thought to inhibit v-src (Whitesell et al. 1992), but was later found to interact with Hsp90 and inhibit formation of the Hsp90/v-src complex (Whitesell et al. 1994). Subsequent studies into the mechanism driving this activity determined geldanamycin to be an ATP-competitive inhibitor (Prodromou et al. 1997). Since then, derivatives of geldanamycin, the macbecins (an additional ansamycin antibiotic) (Bohen 1998; Martin et al. 2008) and several related scaffolds were shown to compete with ATP, and subsequently, inhibit Hsp90 activity. Such selectivity helped propel derivatives of geldanamycin, 17-AAG, 17-DMAG, and IPI-504 (Fig. 9.2) into clinical trials as anti-cancer therapies (reviewed in (Jhaveri et al. 2012)). Due to hepatotoxicity issues associated with this scaffold, which is independent of Hsp90 inhibitory activity, clinical trials have ceased with use of the ansamycin scaffold. However, the use of geldanamycin derivatives have been extensively used outside the clinic to enhance our understanding of Hsp90 biology, particularly in disease. Using this class of Hsp90 inhibitor, it was demonstrated mutant and wild-type proteins have distinct fates following the loss of Hsp90 function. A classic example is the p53 oncogene. Inhibition of Hsp90 promotes the degradation of mutant p53 (Blagosklonny et al. 1996), as mutant p53 is thermodynamically unstable and is therefore more reliant upon the activity of Hsp90 to maintain stability. Wild-type p53, however, remains relatively unaffected following inhibition of Hsp90. As mentioned previously, 17-AAG and geldanamycin were used in seminal studies by Kamal et al. to demonstrate that Hsp90 in cancer cells exhibits higher affinity and distinct co-chaperone complexes as compared to the Hsp90 from non-transformed cells. Although poorly understood, his phenomenon is still a focal point for Hsp90 research and drug development and will be discussed later.

Radicicol (Fig. 9.2), a macrocyclic lactone, is an antibiotic that also acts as an ATP-competitive inhibitor of Hsp90 (reviewed in (Sharp and Workman 2006)). Poor metabolic properties have rendered radicicol inactive in animal studies, and prompted the development of derivatives to improve in vivo activity (Agatsuma et al. 2002; Soga et al. 2001). Radicicol, unlike geldanamycin, does not produce hepatotoxicity (Agatsuma et al. 2002; Soga et al. 2001). Despite this advance over geldanamycin derivatives, no radicicol-based derivatives have advanced into clinical studies. However, the resorcinol ring present in radicicol has been the subject of successful research and clinical advancement and has led to exciting developments in Hsp90-isoform selectivity. Synta Pharmaceuticals compound, STA-9090 (ganetespib) (Fig. 9.2), is one example of this class that is currently under clinical investigation. The use of ganetespib by Taipale in the Lindquist Laboratory has demonstrated several key findings via an elegant screening array (Taipale et al. 2012). These studies demonstrated that there are several dependencies that exist with Hsp90 client proteins. By inhibiting Hsp90 with ganetespib, Taipale identified both 'strong' and 'weak' Hsp90 clients, wherein each class contained client proteins that were degraded or aggregated following the loss of Hsp90 function. These studies concluded that the on-rate of client proteins is the main factor in distinguishing strong and weak clients and found no direct evolutionary correlation between kinase families and Hsp90 client preferences.

Additional modifications to the resorcinol ring have allowed for the generation of small molecules capable of selective-inhibition of the cytosolic Hsp90 family member, Hsp90ß, (KUNB31, Fig. 9.2) (Khandelwal et al. 2018). This advance in the generation of small molecule Hsp90 inhibitors will likely produce effective therapeutics that can avoid some of the problems associated with pan-Hsp90 inhibitors. One example is the dependence of the hERG potassium channel on Hsp90α as well as a lack of induction of the heat shock response (Ficker et al. 2003). Non-selective, or pan, Hsp90 inhibitors can yield cardiotoxicity due to this dependence. Another, and perhaps the most interesting, finding from selective inhibitors was the lack of Hsp70 induction following Hsp90ß-specific inhibition (Soga et al. 2001); Hsp70 induction was long considered an inevitable outcome of Hsp90 inhibition. These findings and continued drug development efforts

will allow researchers to distinguish between the cellular effects of individual Hsp90 family members and produce compounds that may be suitable for translation into the clinic.

The first synthetic Hsp90 inhibitor, PU-3 (Fig. 9.2), is a purine-derived ATP-competitive Hsp90 inhibitor (Chiosis et al. 2002). Following the discovery of PU-3, additional purine-based Hsp90 inhibitors were developed, and include MPC-3100 (Fig. 9.2) by Myriad Pharmaceuticals, BIIB021 (Fig. 9.2) by Biogen, as well as PU-H71 (Fig. 9.2) and PU-AD (structure not disclosed) (reviewed in (Jhaveri et al. 2012); http://www.samustherapeutics.com/; https://www.cancer.gov/publications/dictionaries/cancer-drug/def/iodine-i-124-pu-ad; https://clinicaltrials.gov/ct2/show/NCT03371420). This purine class of Hsp90 inhibitors has been successful thus far during clinical investigation. This success is likely due to their selectivity, pharmacology profiles, and the lack of toxicities that are associated with the natural-product based scaffolds. The purine class, primarily PU-H71 and associated radio-labeled derivatives, have provided a new role for N-terminal Hsp90 inhibitors as imaging agents and diagnostic tools (Rodina et al. 2016). As previously discussed, Kamal et al. performed detailed studies showing Hsp90 in cancer cells to manifest a higher affinity for both ATP and ATP-competitive inhibitors as compared to Hsp90 from non-transformed cells. Rodina and Moulick demonstrated that some, but not all of the Hsp90 in cancer cells present an increased affinity for ATP and ATP-competitive inhibitors (Moulick et al. 2011; Rodina et al. 2016). These properties were further used to identify a network of chaperone complexes with Hsp90, which serves as a central hub for formation of these complexes in cancer. These chaperone hubs, termed the 'epichaperome,' could be evaluated and used as a diagnostic and imaging tool through the use of a radio-labeled purine scaffold Hsp90 inhibitor (Rodina et al. 2016). This discovery allows for enhanced patient selection in the use of Hsp90 inhibitors as anti-cancer agent. In addition, Samus Therapeutics has initiated the clinical evaluation of an epichaperome-targeting agent, named PU-AD, which can serve as an epichaperome biomaker tool for Alzheimer's disease (http://www.samustherapeutics.com/; https://www.cancer.gov/publications/dictionaries/cancer-drug/def/iodine-i-124-pu-ad; https://clinicaltrials.gov/ct2/show/NCT03371420).

Despite the advancement of some Hsp90 N-terminal inhibitors into Phase III clinical trials, clinical advancement has been scarce. Though Hsp90 has demonstrated promise as an anti-cancer target, and perhaps for the treatment of Alzheimer's disease, clinical progress has been slow due to the off-target activities manifested by such compounds.

9.3 Hsp90 C-Terminal Inhibitors

Hsp90-mediated hydrolysis of ATP is required for the folding of bound substrates as well as the release of the folded client. Hsp90 must adopt a closed conformation (Ficker et al. 2003) to undergo ATP hydrolysis. Activator of Hsp90 ATPase activity, Aha1, must first bind Hsp90 before ATP, in an effort to stimulate closure into the closed state. Subsequent structural reorganization ensues, resulting in closure of the N-terminal lid. This reorganization of the conformation represents the rate-limiting step during the protein folding cycle (Prodromou 2012).

It has been demonstrated that the binding of Aha1 to Hsp90 accelerates the conformational change and results in assembly of the closed state. Therefore, small molecules that perturb interactions between Hsp90 and Aha1 are responsible for eliciting unique inhibitory activities that are significantly different than those observed with N-terminal inhibitors.

The coumermycin antibiotics, which include novobiocin (Fig. 9.3), chlorobiocin, and coumermycin A were proposed to bind the N-terminal nucleotide-binding pocket due to structural similarities between the Hsp90 and DNA gyrase ATP-binding sites, both of which contain a unique Bergaret fold. In fact, novobiocin bound competitively versus radicicol and geldanamycin during Hsp90 binding studies. However, neither geldanamycin nor radicicol could displace novobiocin binding to Hsp90 (Marcu et al. 2000).

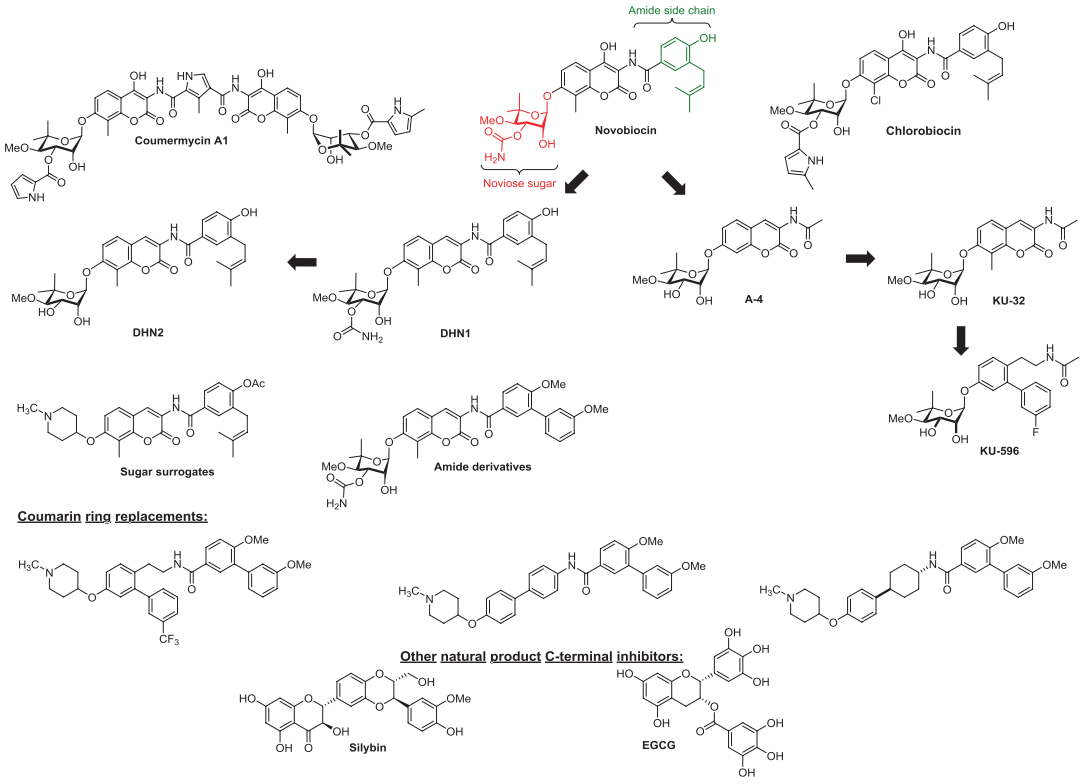


Fig. 9.3 Hsp90 C-terminal inhibitors

Truncated forms of Hsp90 were then evaluated by Neckers and coworkers, who ultimately demonstrated that geldanamycin bound to the N-terminal fragment, but novobiocin bound to a previously unrecognized nucleotide-binding pocket in the C-terminal domain (Marcu et al. 2000). In fact, it was demonstrated that small molecule binding to the C-terminus exhibited allosteric control over the N-terminal binding site.

While novobiocin served as the first Hsp90 C-terminal inhibitor identified, it manifested poor activity in cellular models (Kd ~700 μM in SkBr3 breast cancer cells). Therefore, structure-activity relationship studies were pursued to elucidate the key features of this molecule as well as to improve upon its poor efficacy. The benzamide side chain of novobiocin was replaced with an acetamide, the coumarin ring was modified to remove the 4-hydroxyl, and the 3′-carbamate on the noviose sugar was omitted (Yu et al. 2005). The resulting compound, A4, induced the degradation of Hsp90-dependent client proteins at ~10 μM concentration in the LNCaP prostate cancer cell line (Yu et al. 2005). Remarkably, A4 induced the HSR at concentrations 1000–10,000 fold lower than that needed for client protein degradation (Yu et al. 2005). Prior studies with other classes of Hsp90 inhibitors had never led to segregation of the HSR and client protein degradation. Since A4 exhibited heat-shock induction without the degradation of client proteins, it was evaluated as a neuroprotective agent (Yu et al. 2005). In fact, an analog of KU-32, KU-596, was developed for the treatment of diabetic peripheral neuropathy, and entered clinical trials in 2017. KU-596 is currently awaiting Phase II evaluation.

In contrast to KU-32, which contains the acetamide side chain, 4-deshydroxynovobiocin (DHN1) and 3′-descarbamoyl-4-deshydroxynovobiocin (DHN2) contain the prenylated benzamide side chain, and were prepared to investigate the role of the 4-hydroxyl and

3′-carbamoyl residues (Burlison et al. 2006). Unexpectedly, these compounds did not behave similar to KU-32, and instead induced the degradation of Hsp90-dependent client proteins at concentrations in which no HSR was observed. The most potent compound derived from this series was KU-174, which manifested good inhibitory activity against a large number of cancers in the NCI 60-cell line panel assay (Eskew et al. 2011). Subsequent studies on the coumarin core have been pursued and have led to compounds that exhibit enhanced inhibitory activity and are currently undergoing optimization with the hope of producing an alternative Hsp90 inhibitory class for the treatment of cancer (Kusuma et al. 2014; Donnelly et al. 2008; Bras et al. 2007; Radanyi et al. 2008, 2009).

Based on a number of computational studies, KU-174 was proposed to bind both Hsp90 and Aha1 (Ghosh et al. 2014). Subsequent studies utilizing biotinylated KU-174 demonstrated this molecule indeed binds both Hsp90 and Aha1. However, the aglycone of KU-174 only bound Aha1 (Ghosh et al. 2014), suggesting the noviose sugar is required for binding Hsp90, whereas the aryl amide side chain binds Aha1 (Ghosh et al. 2014). KU-32, which does not contain an aryl amide side chain (acetamide) was biotinylated and also used in affinity purification assays (Eskew et al. 2011). Biotinylated KU-32 was shown to bind both the cytosolic isoform, Hsp90α, and the mitochondria-localized paralog, TRAP-1. Importantly, biotinylated KU-32 did not bind Aha1, supporting the hypothesis that the aryl-containing amide side chain is required for binding Aha1.

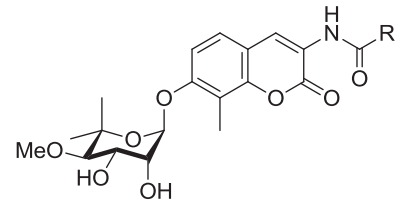
Since KU-32 contains one methyl group on the amide side chain and KU-174 contains a much larger aryl ring on the amide side chain, studies were initiated to identify the point of divergence in which the pro-survival neuroprotective analog was transformed into a compound with anti-cancer activity. Therefore, derivatives of KU-32 were investigated that contained increasingly larger alkyl and cycloalkyl groups on the amide side chain (Table 9.1). The antiproliferative activity manifested by the KU-32 analogs was evaluated against both SkBr3 breast and the androgen independent PC3-MM2 prostate cancer cell lines. Upon evaluation of the results, it became clear that increasing the alkyl chain length resulted in a size-dependent increase in antiproliferative activity as shown in Table 9.1, which was linearly correlative with chain length or bulk.

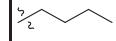
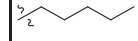
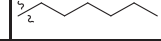
Since the rematuration of firefly luciferase is dependent upon Hsp90, the refolding of denatured firefly luciferase was used to determine whether these analogs affected this process (Matts et al. 2011; Galam et al. 2007; Davenport et al. 2014; Avila et al. 2006). KU-32 did not inhibit the ability of Hsp90 to refold luciferase, but analogs containing the longer alkyl chains or cyclic alkanes did inhibit the re-maturation of firefly luciferase (Fig. 9.4). However, analogs containing shorter alkyl chains failed to inhibit the re-maturation of luciferase, but in contrast to the larger substituents, the smaller alkyl groups increased the re-maturation of firefly luciferase. One explanation that could account for the divergence in activities is that Aha1 remains bound to Hsp90 when the amide side chain is small, however, in the presence of larger side chains, the interaction between Hsp90 and Aha1 is disrupted. Co-immunoprecipitation experiments were performed in the presence of these analogs and it was demonstrated that Hsp90α/Aha1 disruption occurred more readily for the amide side chains that contained larger alkyl groups, which resulted in a linear correlation between activity and chain length (Fig. 9.4).

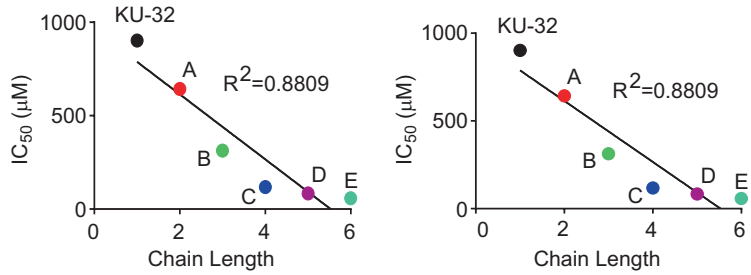
(−)-Epigallocatechin-3-gallate (EGCG) and silybin have been shown to bind the Hsp90 C-terminus and to modulate the Hsp90-mediated protein folding machinery. Gasiewicz and coworkers showed EGCG to bind the same amino acids (538–728) as novobiocin via proteolytic footprinting, immunoprecipitation, and an ATP-argarose pull-down assay (Yin et al. 2009). Inhibition of Hsp90 with EGCG was shown to induce the degradation telomerase, kinases, and the aryl hydrocarbon receptor, and consequently, manifests anti-cancer activity (Palermo et al. 2005; Khandelwal et al. 2013).

Silybin is a traditional medicine that has been used to treat liver and gallbladder disorders (Lu

Table 9.1 Antiproliferative activity resulting from increasing tail length



Entry	R	SKBr3 (IC_{50}, μM)	PC3-MM2 (IC_{50}, μM)
KU32	-Me	902	>500
A		643 ± 50	504
B		313 ± 9	466
C		118 ± 43	317
D		84 ± 38	53.7 ± 0.35
E		58.3 ± 2.1	34.7 ± 1.82
F		517 ± 29	>500
G		220 ± 16	284 ± 99.85
H		67.0 ± 1.4	144.5 ± 10.6
I		46.8 ± 0.7	54.55 ± 4.92



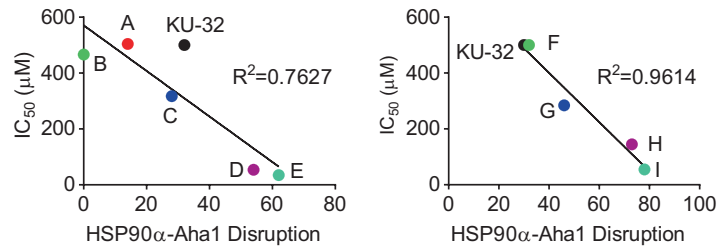


Fig. 9.4 KU-32 analogs disrupt the Hsp90α/Aha1 complex. IC_{50} values report inhibition of refolding of firefly luciferase

et al. 2009). However, silybin was also shown to manifest cytotoxic activity against various cancer cell lines In addition to enhancing the efficacy of chemotherapeutic agents, silybin also manifests anti-cancer activity against a number of cancer cell lines. Silybin has also been shown to possess Hsp90 C-terminal inhibitory activity and cause Hsp90-dependent client proteins degradation without alteration of Hsp90 levels (Zhao et al. 2011). Due to the similar biological properties between novobiocin and silybin, chimeric derivatives of these two natural products were developed, which led to compounds with improved Hsp90 inhibitory activity (Zhao et al. 2012).

9.4 Disruption of Hsp90-co-chaperone Interactions

Hsp90 and its co-chaperones are required for the conformational maturation of the majority of Hsp90-dependent substrates. Below are examples of natural products that have been discovered to disrupt these interactions and consequently, produce activities that are different than those observed with both N- and C-terminal inhibitors (Fig. 9.5). For example, celastrol disrupts interactions between Hsp90 and Cdc37, which is a co-chaperone required for the proper folding of Hsp90-dependent kinases (Zhang et al. 2009). Thus, celastrol manifests its Hsp90

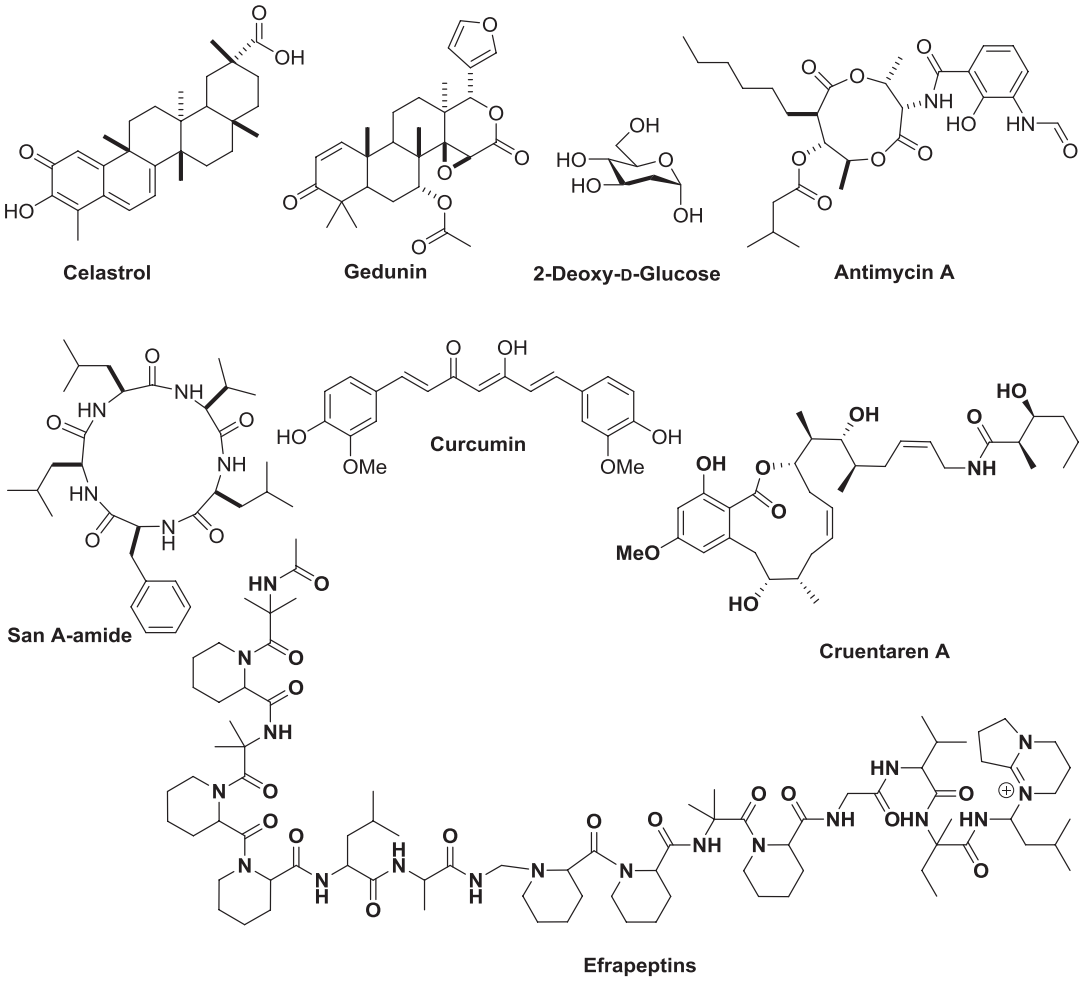


Fig. 9.5 Small molecules that disrupt formation of the Hsp90 heteroprotein complex

inhibitory activity through preventing the maturation of kinases, with little effect on other Hsp90-dependent substrates. The natural product gedunin disrupts Hsp90/p23 interactions, which is a co-chaperone required for the stabilization of Hsp90's closed state, and is required for the maturation of various client proteins (Patwardhan et al. 2013). Interestingly, administration of gedunin to cells does not induce Hsp27 levels, suggesting this approach may not induce the pro-survival HSR.

Since Hsp90 requires ATP for its protein folding activity, the inhibition of ATP synthase with ATP synthase inhibitors including oligomycin A, 2-deoxy-D-glucose, antimycin A and efrapeptins, prevent the Hsp90-dependent maturation of substrates via destabilization of the client-Hsp90 complex, leading to client degradation via the proteosome (Papathanassiu et al. 2011; Hall et al. 2014; Peng et al. 2005). In addition, ATP synthase inhibitors do not induce the HSR, and both oligomycin A and the efrapeptins manifest little to no increase in Hsp90, Hsp70 nor Hsp27 levels. More recently, inhibition of F_1F_0 ATP synthase with the only known selective inhibitor, cruentaren A, was shown to induce client protein degradation through destabilization of the F_1F_0 ATP synthase-Hsp90α interaction (Papathanassiu et al. 2011; Kunze et al. 2007; Jundt et al. 2006). Hsp90 and p23 also form a complex with hTERT, the catalytic subunit of telomerase, a protein whose function contributes to unlimited replicative potential of cancer cells. Curcumin, the active component of Indian curry, was shown to induce the degradation of hTERT through disruption of p23-hTERT interactions as one of its many mechanisms of action (Lee and Chung 2010). Interestingly, curcumin disrupted interactions between p23 and hTERT, but did not alter hTERT's affinity for Hsp90. For comparison, the N-terminal Hsp90 inhibitor, geldanamycin, disrupts both the Hsp90-hTERT and p23-hTERT complexes, leading to the degradation of hTERT.

A derivative of the natural product, sansalvamide A, san A-amide, was demonstrated to induce apoptosis in pancreatic, colon, breast and prostate cancer cell lines (Ardi et al. 2011; Mcconnell et al. 2014). Upon further evaluation, it was shown that San A-amide induced apoptosis in HCT-116 colon cancer cells through displacement of inositol hexakisphosphate kinase-2 (IP6K2) and FKBP52 from the Hsp90 C-terminus (Vasko et al. 2010). It appears as though san A-amide disrupts the structure of the Hsp90 N-terminus, which then alters the substrate binding site. The observed activities appear distinct from other Hsp90 inhibitory classes, as san A-amide exhibits no effect on Her2. In contrast, the Hsp90 N-terminal inhibitor, 17-allylaminogeldanamycin (17-AAG), does not affect IP6K2 and FKBP52 binding, but does inhibit the maturation/activation of Her2.

9.5 Conclusion

While Hsp90 has been seriously sought after as a chemotherapeutic target for the treatment of cancer, all of the inhibitors evaluated for this disease manifested a similar mechanism of action – inhibition of the N-terminal ATPase. Perhaps, alternative approaches toward Hsp90 inhibition that includes disruption of co-chaperone interactions, inhibition of the Hsp90 C-terminus, or isoform-selective inhibition will overcome some of the detriments associated with pan inhibition of the N-terminal ATP-binding site of Hsp90. Alternatively, through modulation of these other domains and partner proteins, modulation of the Hsp90 chaperone machine may be useful for the treatment of other diseases, much like KU-596, which is undergoing clinical evaluation for neuropathy.

Acknowledgements The authors gratefully acknowledge financial support from the National Institutes of Health (CA120458 and CA213566).

References

Agatsuma T, Ogawa H, Akasaka K, Asai A et al (2002) Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities. Bioorg Med Chem 10:3445–3454

Ardi VC, Alexander LD, Johnson VA, Mcalpine SR (2011) Macrocycles that inhibit the binding between

heat shock protein 90 and TPR-containing proteins. ACS Chem Biol 6(12):1357–1366

Avila C, Hadden MK, Ma Z, Kornilayev BA, Ye QZ, Blagg BS (2006) High-throughput screening for Hsp90 ATPase inhibitors. Bioorg Med Chem Lett 16:3005–3008

Blagosklonny MV et al (1996) Mutant conformation of p53 translated in vitro or in vivo requires functional HSP90. Proc Natl Acad Sci U S A 93:8379–8383

Bohen SP (1998) Genetic and biochemical analysis of p23 and ansamycin antibiotics in the function of Hsp90-dependent signaling proteins. Mol Cell Biol 18(6):3330–3339. https://doi.org/10.1128/MCB.18.6.3330

Bras GL, Radanyi C, Peyrat J-F et al (2007) New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90. J Med Chem 50:6189–6200

Burlison JA, Neckers L, Smith AB, Maxwell A, Blagg BSJ (2006) Novobiocin: redesigning a DNA gyrase inhibitor for selective inhibitor of Hsp90. J Am Chem Soc 128:15529–15536

Chiosis G, Lucas B, shtil A, Huezo H, Rosen N (2002) Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. Bioorg Med Chem 10(11):3555–3564

Davenport J, Balch M, Galam L, Girgis A, Hall J, Blagg BS, Matts RL (2014) High-throughput screen of natural product libraries for hsp90 inhibitors. Biology 3:101–138

DeBoer C, Meulman PA, Wnuk RJ, Peterson DH (1970) Geldanamycin, a new antibiotic. J Antibiot (Tokyo) 23:442–447

Dickey CA, Kamal A, Lundgren K et al (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest 117(3):648–658

Donnelly AC, Mays JR, Burlinson JA et al (2008) The design, synthesis and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity. J Org Chem 73:8901–8920

Eskew JD, Sadikot T, Morales P et al (2011) Development and characterization of a novel C-terminal inhibitor of Hsp90 in androgen dependent and independent prostate cancer cells. BMC Cancer 11:468

Ficker E, Dennis AT, Wang L, Brown AM (2003) Role of the cytosolic chaperones Hsp70 and Hsp90 in maturation of the cardiac potassium channel hERG. Circ Res 92(12):e87–e100

Galam L, Hadden MK, Ma Z, Ye QZ, Yun BG, Blagg BS, Matts RL (2007) High-throughput assay for the identification of Hsp90 inhibitors based on Hsp90-dependent refolding of firefly luciferase. Bioorg Med Chem 15:1939–1946

Ghosh S, Shinogle HE, Garg G, Vielhauer GA et al (2014) Hsp90 C-terminal inhibitors exhibit antimigratory activity by disrupting the Hsp90α/Aha1 complex in PC3-MM2 cells. ACS Chem Biol 10(2):577–590

Hall JA, Kusuma BR, Brandt GEL, Blagg BSJ (2014) Cruentaren a binds F1F0 ATP synthase to modulate the Hsp90 protein folding machinery. ACS Chem Biol 9:976–985

Jhaveri K, Taldone T, Modi S, Chiosis G (2012) Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancer. Biochim Biophys Acta 1823(3):742–755

Jundt L, Steinmetz H, Luger P et al (2006) Isolation and structure elucidation of cruentarens A and B – novel members of the benzolactone class of ATPase inhibitors from the myxobacterium Byssovorax cruenta. Eur J Org Chem 2006(22):5036–5044

Kamal A, Thao L, Sensintaffar J et al (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425(6956):407–410

Khandelwal A, Hall JA, Blagg BS (2013) Synthesis and structure-activity relationships of EGCG analogues, a recently identified Hsp90 inhibitor. J Org Chem 78(16):7859–7884

Khandelwal A, Kent CN, Balch M et al (2018) Structure-guided design of an Hsp90ß N-terminal isoform-selective inhibitor. Nat Commun 9(1):425

Kunze B, Sasse F, Wieczorek H, Huss M (2007) Cruentaren a, a highly cytotoxic benzolactone from myxobacteria is a novel selective inhibitor of mitochondrial F1-ATPases. FEBS Lett 581(18):3523–3527

Kusuma BR, Khandelwal A, Gu W et al (2014) Synthesis and biological evaluation of coumarin replacements of novobiocin as Hsp90 inhibitors. Bioorg Med Chem 22(4):1441–1449

Lee JH, Chung IK (2010) Curcumin inhibits nuclear localization of telomerase by dissociating the Hsp90 co-chaperone p23 from hTERT. Cancer Lett 290(1):76–86

Lu P, Mamiya T, Lu LL et al (2009) Silibinin attenuates amyloid beta(25–35) peptide-induced memory impairments: implication of inducible nitric-oxide synthase and tumor necrosis factor-alpha in mice. J Pharmacol Exp Ther 331(1):319–326

Marcu MG, Schulte TW, Neckers L (2000) Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. J Natl Cancer Inst 92(3):242–248

Martin CJ, Gaisser S, Challis IR, Wilkinson B, Gregory M, Prodromou C, Roe SM, Pearl LH, Boyd SM, Zhang MQ (2008) Molecular characterization of macbecin as an Hsp90 inhibitor. J Med Chem 51(9):2853–2857. https://doi.org/10.1021/jm701558c. Epub 2008 Mar 22

Matts RL, Brandt GE, Lu Y et al (2011) A systematic protocol for the characterization of Hsp90 modulators. Bioorg Med Chem 19:684–692

Mcconnell JR, Alexander LA, Mcalpine SR (2014) A heat shock protein 90 inhibitor that modulates the immunophilins and regulates hormone receptors without inducing the heat shock response. Bioorg Med Chem Lett 24(2):661–666

Moulick K, Ahn JH, Zong H, Rodina A et al (2011) Affinity-based proteomics reveal cancer-specific

networks coordinated by Hsp90. Nat Chem Biol 7(11):818–826

Palermo CM, Westlake CA, Gasiewicz TA (2005) Epigallocatechin gallate inhibits aryl hydrocarbon receptor gene transcription through an indirect mechanism involving binding to a 90 kDa heat shock protein. Biochemistry 44:5041–5052

Papathanassiu A, Macdonald N, Emlet D, Vu H (2011) Antitumor activity of efrapeptins, alone or in combination with 2-deoxyglucose, in breast cancer in vitro and in vivo. Cell Stress Chaperones 16(2):181–193

Patwardhan CA, Fauq A, Peterson LB, Miller C, Blagg BSJ, Chadli A (2013) Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. J Biol Chem 288(10):7313–7325

Peng X, Guo X, Borkan SC et al (2005) Heat shock protein 90 stabilization of ErbB2 expression is disrupted by ATP depletion in myocytes. J Biol Chem 280(13):13148–13152

Prodromou C (2012) The 'Active Life' of Hsp90 complexes. Biochim Biophys Acta 1823(3):614–623

Prodromou C, Roe SM, O'brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90(1):65–75

Radanyi C, Bras GL, Messaoudi S et al (2008) Synthesis and biological activity of simplified denoviosecoumarins related to novobiocin as potent inhibitors of heat-shock protein 90 (hsp90). Bioorg Med Chem Lett 18(7):2495–2498

Radanyi C, Bras GL, Marsaud V et al (2009) Antiproliferative and apoptotic activities of tosylcyclonovobiocic acids as potent heat shock protein 90 inhibitors in human cancer cells. Cancer Lett 274(1):88–94

Rodina A, Wang T, Yan P et al (2016) The epichaperome is an integrated chaperome network that facilitates tumor survival. Nature 538(7625):397–410

Sharp S, Workman P (2006) Inhibitors of HSP90 molecular chaperone: current status. Adv Cancer Res 95:323–348

Soga S, Sharma SV, Shiotsu Y et al (2001) Stereospecific antitumor activity of radicicol oxime derivatives. Cancer Chemother Pharmacol 48:435–445

Taipale M, Krykbaeva I, Koeva M et al (2012) Quantitative analysis of Hsp90-client interactions reveals principles of substrate recognition. Cell 150(5):987–1001

Vasko RC, Rodriguez RA, Cunningham CN, Ardi VC, Agard DA, Mcalpine SR (2010) Mechanistic studies of sansalvamide A-amide: an allosteric modulator of Hsp90. ACS Med Chem Lett 1(1):4–8

Whitesell L, Shifrin SD, Schwab G, Neckers LM (1992) Benzoquinonoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res 52:1721–1728

Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM (1994) Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A 91:8324–8328

Yin Z, Henry EC, Gasiewicz TA (2009) (−)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. Biochemistry 48:336–345

Yu XM, Shen G, Neckers L et al (2005) Hsp90 inhibitor identified from a library of novobiocin analogues. J Am Chem Soc 127:12778–12779

Zhang T, Li Y, Yu Y, Zou P, Jiang Y, Sun D (2009) Characterization of celastrol to inhibit Hsp90 and Cdc37 interaction. J Biol Chem 284(51):35381–35389

Zhao H, Brandt GE, Galam L, Matts RL, Blagg BSJ (2011) Identification and initial SAR of silybin: an Hsp90 inhibitor. Bioorg Med Chem Lett 21(9):2659–2664

Zhao H, Yan B, Peterson LB, Blagg BSJ (2012) 3-arylcoumarin derivatives manifest anti-proliferative activity through Hsp90 inhibition. ACS Med Chem Lett 3(4):327–331

10 Lessons Learned from Proteasome Inhibitors, the Paradigm for Targeting Protein Homeostasis in Cancer

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Abstract

Targeting aberrant protein homeostasis (proteostasis) in cancer is an attractive therapeutic strategy. However, this approach has thus far proven difficult to bring to clinical practice, with one major exception: proteasome inhibition. These small molecules have dramatically transformed outcomes for patients with the blood cancer multiple myeloma. However, these agents have failed to make an impact in more common solid tumors. Major questions remain about whether this therapeutic strategy can be extended to benefit even more patients. Here we discuss the role of the proteasome in normal and tumor cells, the basic, preclinical, and clinical development of proteasome inhibitors, and mechanisms proposed to govern both intrinsic and acquired resistance to these drugs. Years of study of both the mechanism of action and modes of resistance to proteasome inhibitors reveal these processes to be surprisingly complex. Here, we attempt to draw lessons from experience with proteasome inhibitors that may be relevant for other compounds targeting proteostasis in cancer, as well as extending the reach of proteasome inhibitors beyond blood cancers.

Keywords

Proteasome · Bortezomib · Carfilzomib · Myeloma · Proteasome inhibitor · Resistance · Protein homeostasis · Unfolded protein response

10.1 Introduction

One of the hallmarks of tumor cells is greatly increased protein synthesis when compared to normal, non-transformed cells (Laplante and Sabatini 2012; Harper and Bennett 2016). This increased protein production capacity is necessary to support the rapid proliferative state within a tumor. However, this phenomenon of "proteome imbalance" (Harper and Bennett 2016) may also create novel therapeutic opportunities. For example, directly targeting protein synthesis via the inhibition of translation initiation is a promising approach to selectively eliminate tumor cells (Wolfe et al. 2014). Alternatively, and as discussed extensively in other chapters here, many protein-folding chaperones are also found to be upregulated across cancers compared to

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_10

normal cells and may serve as selective vulnerabilities (Chatterjee and Burns 2017).

Furthermore, it is now well-understood that this increased protein synthetic burden leads to a large fraction of newly-synthesized proteins not achieving their final, native state (Harper and Bennett 2016). Unfolded proteins accumulating within the cell lead to proteotoxic stresses that can impair cellular viability. The mechanism of the unfolded protein response (UPR) has been particularly well-characterized, where unfolded proteins accumulating in the endoplasmic reticulum first lead to an adaptive response to proteotoxic stress but, if the stress cannot ultimately be resolved, to cellular apoptosis (Walter and Ron 2011).

Both for normal cellular homeostasis as well as to resolve proteotoxic stress in tumor cells, mechanisms to degrade unfolded proteins and recycle them to their constituent amino acids for synthesis of new proteins are central to viability. There are two major pathways for protein degradation. The first is the ubiquitin-proteasome system, which appears responsible for degradation of ~80% of cellular protein under normal conditions (Zhao et al. 2015) and will be discussed in more detail below. The second is the autophagy pathway, which is not discussed further here but has recently been reviewed in detail by others (Kenific and Debnath 2015; Mizushima and Komatsu 2011; Levy et al. 2017).

10.2 Defining the Target: Structure and Function of the Proteasome

Given its central role in disposing of cellular proteins, the proteasome is often characterized as the "garbage can" of the cell. Its structure lends itself to this analogy. The core 20S proteasome is composed of 7 homologous α subunits, comprising the two symmetrical outer rings, and 7 homologous β subunits, comprising the two symmetrical inner rings, arranged into a cylindrical structure (Fig. 10.1a–b). Three of the β subunits are responsible for the proteolytic activity of the proteasome: the β1, β2, and β5 subunits (encoded by the genes *PSMB1*, *PSMB2*, and *PSMB5*, respectively). These subunits are able to cleave proteins with caspase-like, trypsin-like, and chymotrypsin-like specificities, respectively. Substrate proteins are typically degraded to short polypeptides in the range of 3–25 amino acids (Nussbaum et al. 1998), which can then be further recycled to individual amino acids by endopeptidases (Saric et al. 2004) or loaded onto the major histocompatibility complex proteins for antigen presentation to the immune system (Sijts and Kloetzel 2011). Notably, proteasomes are present at high levels both in the cytosol and the nucleus (von Mikecz 2006).

Targeted degradation by the proteasome itself is intimately linked to post-translational modification of protein substrates by poly-ubiquitin chains. The ubiquitination pathway is complex and will only be briefly discussed here (we refer readers to prior extensive reviews (Hershko and Ciechanover 1998; Ravid and Hochstrasser 2008; Kleiger and Mayor 2014)), but it rests on the transfer of the small, globular protein ubiquitin from an initial E1 "activating" enzyme, of which there are only 2 encoded in the genome, to an E2 ubiqutin conjugating enzyme (~40 individual genes) and ultimately to an E3 ubiquitin ligase (>600 individual genes) with targeted substrate specificity (Kleiger and Mayor 2014). While post-translational substrate modification with ubiquitin can lead to a range of biological effects, poly-ubiquitin chains linked at lysine 48 are considered the classic signal for degradation via the proteasome. In addition to its role in degrading the majority of folded proteins, it is estimated that this ubiquitin-proteasome system (UPS) may rapidly and co-translationally degrade up to 30% of newly-synthesized proteins due to defective folding or other errors (Schubert et al. 2000).

The proteasome is most often pictured as the "full" 26S proteasome, composed of both the 20S core particle and capped by either one or two 19S regulatory particles (Fig. 10.1a). While the structure of the 20S core particle has been well-known

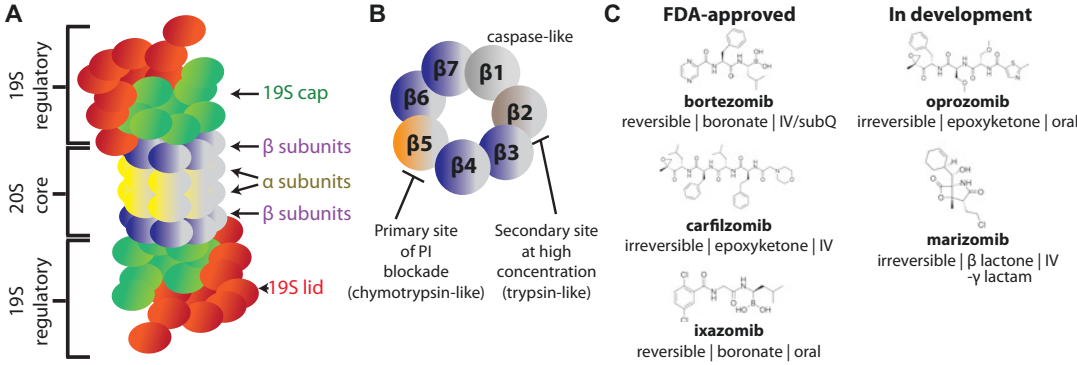

Fig. 10.1 Structure and inhibition of the proteasome. (**a**). Cartoon structure of the 26S proteasome including the 20S core particle, consisting of symmetrical α and β subunit rings, as well as the 19S regulatory particle including both the cap subassembly, directly bound to the core particle, and the lid subassembly. (**b**). Catalytic β subunits and inhibition by FDA-approved proteasome inhibitors. (**c**). Clinically relevant proteasome inhibitors including FDA approval status, reversibility of inhibition, chemical scaffold, and route of administration

for years, recent advances in cryogenic electron microscopy (cryo-EM) have begun to reveal the detailed structure of the full 26S proteasome including the 19S cap (Sledz and Baumeister 2016; Dong et al. 2019; Huang et al. 2016; Lander et al. 2012; Lasker et al. 2012). In yeast and humans, the 19S cap is composed of up to 19 individual proteins divided between two-subassemblies, a 10-unit base and a 9-unit lid. Subunits include those responsible for engaging ubiqutinated substrates (Rpn1 (*PSMD2*)/Rpn2 (*PSMD1*)), binding to polyubiquitin chains (Rpn10 (*PSMD4*)/Rpn13 (*ADRM1*)) and then progressively removing ubiquitin molecules (Rpn11 (*PSMD14*): the 19S "de-ubiquitinase" or "DUB") (Sledz and Baumeister 2016; Tanaka 2009). These 19S subunits interact with the substrate while it is mechanically unfolded and simultaneously guided into the pore at the center of the 20S core for processive proteolysis.

Notably, though, in typical cellular settings not all proteasomes are in a stable complex with the 19S cap (Peters et al. 1993; Yoshimura et al. 1993). In other conformations, the 20S core may instead interact with a smaller bleomycin-sensitive 10S cap or an 11S cap which do not require ubiquitination or ATP for degradation (Cromm and Crews 2017). While the physiological role of these alternate forms of the proteasome, or ubiquitin-independent degradation in general, remain unclear, these mechanisms may degrade a large fraction of proteins (Kish-Trier and Hill 2013; Rechsteiner and Hill 2005; Erales and Coffino 2014). One observation is that ubiquitin-independent degradation may be triggered by interaction with disordered protein regions (Erales and Coffino 2014; Baugh et al. 2009; Jariel-Encontre et al. 2008).

Another important alternate form of the proteasome is the "immunoproteasome". This form of the proteasome incorporates different proteolytic beta-subunits (β1i, β2i, and β5i subunits instead of β1, β2, and β5 subunits) within the core 20S structure. The immunoproteasome is preferentially expressed in cells of the immune system, induced by interferon-γ, and appears to result in different cleavage patterns of peptides presented via MHC class I (Sijts and Kloetzel 2011; Boes et al. 1994; Ustrell et al. 1995; Winter et al. 2017). Notably, it is hypothesized that specific inhibitors of the immunoproteasome may be advantageous in targeting immune-origin cells and avoiding toxicity in other cell types. For example, this approach is being actively explored not only in blood cancers but also in the setting of autoimmune disease (Ettari et al. 2016).

10.3 Overcoming Skepticism: Preclinical Development of Proteasome Inhibitor Therapeutics

One of the remarkable aspects of the proteasome inhibitors (PIs) is that they were ever developed at all. Significant skepticism surrounded the tractability of the proteasome as a therapeutic target given its essential role in cellular homeostasis. In fact, PIs were not initially developed as cancer therapeutics. Instead, the first PIs were developed by the start-up MyoGenics to treat muscle wasting disorders (Goldberg 2012). One of their initial lead compounds, MG-132, also inhibited other proteases in addition to the β5 subunit of the proteasome (Adams et al. 1999). MG-132 was therefore abandoned as a clinical candidate, though it has had a remarkable second life as a research tool. However, the boronic acid derivative bortezomib (initially known as MG-341, later PS-341) showed more favorable specificity for inhibiting the chymotrypsin-like activity of the proteasome. While bortezomib was not successful for the initial indication of muscle wasting, screening of bortezomib versus the NCI-60 cancer cell line panel revealed that the large majority of tumor cell lines showed strong sensitivity to this agent, with the average LC_{50} of only 7 nM (Adams 2002). In contrast, several types of non-neoplastic cells showed much lower sensitivity to bortezomib (Hideshima et al. 2001).

These initial findings raised interest in PIs as potential therapeutics, as these preclinical data suggested a real therapeutic index – i.e. the ability to kill tumor cells while sparing normal cells – for treatment of cancer patients. Subsequent preclinical studies showed efficacy of bortezomib against a wide range of human tumor cell line xenografts in immunocompromised mice including both solid tumors and hematologic malignancies (Adams 2002). These studies also noted the reversible nature of bortezomib and suggested initial dosing schedules for clinical trials.

10.4 Moving Proteasome Inhibitors from the Bench to the Clinic

These promising preclinical studies quickly motivated clinical trials of bortezomib against numerous solid and hematologic cancers (Goldberg 2012; Roeten et al. 2018). Unfortunately, results were generally disappointing. Minimal, if any, single agent activity was observed in heavily pre-treated solid tumor patients (Huang et al. 2014). In the face of these setbacks, however, there was a critical glimmer of hope. In a Phase I study, a single relapsed/refractory multiple myeloma (RRMM) patient showed a complete response (CR) in response to bortezomib monotherapy (Goldberg 2012). This remarkable response in a disease with (at the time) a uniformly miserable prognosis was a spark for both optimism and intense investigation.

Additional studies in MM quickly followed. Food and Drug Administration (FDA) approval of bortezomib for RRMM was granted in 2003 based on the results of the Phase II SUMMIT (Richardson et al. 2003) and CREST (Jagannath et al. 2004) studies. These findings were strengthened by the Phase III APEX trial, where bortezomib was shown to significantly improve response rate, time to progression, and survival in RRMM compared to standard therapy (Richardson et al. 2005). Numerous additional Phase III studies have demonstrated bortezomib to be an effective component of combination therapies in either the up-front or relapsed settings (San Miguel et al. 2008; Dimopoulos et al. 2015; Spencer et al. 2018a; Durie et al. 2017). Bortezomib is now regarded as a cornerstone of MM therapy, playing a major role in transforming MM from a death sentence to a chronic disease for many patients (Choudhry et al. 2018).

Notably, clinical trials in mantle cell lymphoma (MCL) (Fisher et al. 2006), a poor-prognosis B-cell lymphoma, showed improved survival with bortezomib, leading to FDA

approval in 2007 for relapsed/refractory MCL. Clinical results in Waldenström's macroglobulinemia, a B-cell malignancy characterized by high levels of IgM production, as well as AL amyloidosis, a malignancy of plasma cells similar in pathogenesis to MM, also showed strong response to bortezomib (Treon et al. 2009; Reece et al. 2009) though these indications have not yet been FDA-approved.

However, bortezomib is not curative for MM (and neither is any other currently-available MM therapy (Choudhry et al. 2018)). The development of acquired resistance to bortezomib is common, and treatment is often limited by dose-limiting side effects. Even with the advent of subcutaneous bortezomib dosing to minimize significant peripheral neuropathy, this toxicity is still experienced by 5–10% of patients (Petrucci et al. 2014). These limitations spurred the development of additional PIs, two of which are now FDA-approved for RRMM: carfilzomib (approved in 2012) (Leleu et al. 2019), an intravenous-administered epoxyketone derivative that is an irreversible inhibitor of the 20S β5 subunit (Kuhn et al. 2007; O'Connor et al. 2009), and ixazomib (approved in 2015) (Dimopoulos et al. 2017a), an orally-bioavailable, boronate-based, reversible inhibitor of β5 (Chauhan et al. 2011; Kupperman et al. 2010). We do note that at high, potentially non-physiological, concentrations, β2 and possibly β1 can also be inhibited by all the above PIs (Besse et al. 2019; de Bruin et al. 2016; Kraus et al. 2015). Several other PIs are also in development, with the natural product derivative marizomib and oral epoxyketone oprozomib the farthest in trials (Gandolfi et al. 2017) (Fig. 10.1c).

Consistent with the hypothesis around its design, irreversible inhibition of the proteasome by carfilzomib drove deeper clinical remissions than bortezomib (Dimopoulos et al. 2017a; Dimopoulos et al. 2016). Furthermore, preclinical studies (Kuhn et al. 2007; Demo et al. 2007) and clinical data (Stewart et al. 2015; Siegel et al. 2018) showed that MM refractory to bortezomib could respond to carfilzomib as second-line therapy, further supporting that these two agents are not equivalent. Notably, carfilzomib also does not lead to appreciable amounts of peripheral neuropathy, though its most prominent toxicity is cardiac (Waxman et al. 2018; Dimopoulos et al. 2017b). The development of carfilzomib was also spurred by the hypothesis that irreversible, deeper proteasome inhibition may overcome bortezomib's shortcomings in solid tumors. However, initial trials of carfilzomib in other indications have shown limited antitumor activity (Papadopoulos et al. 2013).

In terms of other PIs, differential effects of oral ixazomib and oprozomib from their intravenously-administered counterparts (bortezomib and carfilzomib, respectively) remain to be elucidated. Notably, mirazomib is the only PI under clinical development with a backbone not based on a peptide analog (O'Connor et al. 2009). This unique structure leads to similar inhibition of both β5 and β2 subunits of the 20S core particle. Preclinical data have indicated slightly different mechanisms of inducing MM cell death than bortezomib (Chauhan et al. 2005). Initial clinical trials in MM have been promising (Spencer et al. 2018b). It will be intriguing to evaluate whether this agent carries promise for other non-MM indications, particularly in light of one recent study suggesting that co-targeting β2 with selective inhibitors greatly increased sensitivity of triple negative breast cancer cell lines to bortezomib (Weyburne et al. 2017).

10.5 Why Are Proteasome Inhibitors Effective? Proposed Mechanisms of Action

Following initial preclinical studies demonstrating increased sensitivity of cancer cells to bortezomib relative to non-malignant cells, a number of groups explored potential mechanisms of action for PIs. One initial hypothesis revolved

around disruption of cancer cell proliferation via stabilization of cell cycle checkpoint inhibitors typically degraded by the proteasome (Strauss et al. 2007). Others suggested that as cancer cells tend to express higher levels of the proteasome subunits, they may be more "addicted" to the proteasome for survival and thereby more sensitive to inhibitors (Tsvetkov et al. 2018). Another hypothesis, discussed in more detail below, is that increased protein synthesis in cancer cells, and a resulting increase is misfolded proteins, leaves cancer cells more reliant on the proteasome to mitigate proteotoxic stress (Bianchi et al. 2009; Cenci et al. 2012).

Given the widespread clinical usage of bortezomib in MM, the majority of PI mechanism of action studies have focused on this disease. Given early basic research suggesting the proteasome is critical for degradation of the NF-κB inhibitor IκB (Palombella et al. 1994; Traenckner et al. 1994), initial mechanistic work in MM also closely focused on this pathway (Hideshima et al. 2002). This pathway is known to be highly active in many tumors and is important for governing cell survival and proliferation. Indeed, among cancer types, MM appears to be among the most dependent on NF-κB for survival (Matthews et al. 2016). However, data demonstrating differential sensitivity of cell lines to PIs and NF-κB inhibitors have suggested that NF-κB is unlikely to be the sole PI mechanism of action (Matthews et al. 2016; Cvek and Dvorak 2011).

Other hypotheses for the PI mechanism of action have also focused on degradation dynamics of cancer-relevant proteins. Some studies have suggested that blocking the proteasome may preferentially stabilize apoptosis-promoting BH3-family proteins, particularly Bim (Fennell et al. 2008; Li et al. 2008). Another intriguing hypothesis has to do with the downstream output of the proteasome. As mentioned above, the proteasome is essential for replenishing the total cellular pool of amino acids so that new proteins may be synthesized. An intriguing study in cells from three different organisms suggested that proteasome inhibition leads to an amino acid starvation state, thereby triggering death through the integrated stress response pathway (Suraweera et al. 2012). Work from our group has also suggested that interference with pre-mRNA splicing may also represent a downstream effect of PIs to decrease tumor cell fitness (Huang et al. 2020).

One major takeaway from these studies is that PIs do not have a straightforward, single mechanism of action. Perhaps this should be expected when inhibiting a molecular machine that interfaces with the large majority of cellular proteins. However, there is still value in determining what is the primary contributor to the anti-tumor effects of these drugs; this is particularly true when it comes to either extending the reach of PIs to other indications or attempting to overcome drug resistance in currently-treated patients.

To our mind, the most compelling explanations for the PI mechanism of action directly address the clinical finding that bortezomib works extremely well in MM and closely-related malignancies, but has been largely ineffective in solid tumors. Notably, analysis of cancer cell line data in the Genomics of Drug Sensitivity in Cancer database, which profiled bortezomib sensitivity across >400 cell lines, found that MM cells are actually not the most sensitive to proteasome inhibition (Fig. 10.2a). Melanoma skin cancer lines in fact appear the most responsive, followed by renal carcinoma and glioblastoma. Additionally, our analysis of CRISPR screen data in the Cancer Dependency Map project (Tsherniak et al. 2017) showed that cell lines derived from several other tumor types appear, in aggregate, more sensitive to genetic depletion of core subunits of the proteasome than MM cells (Huang et al. 2020).

However, it is of course important to keep in mind that cell lines do not faithfully represent patient tumors. A major consideration is that cancer cell lines do not include any features of the tumor microenvironment. These include surrounding immune cells as well as supporting mesenchymal cells, such as stromal cells or cancer-associated fibroblasts, that can play major contributions in driving tumor cell growth and governing response to therapy (Bianchi and Munshi 2015; Manier et al. 2016; Liu et al. 2019a). In addition, a primary consideration for all cancer cell lines is that they have differentially

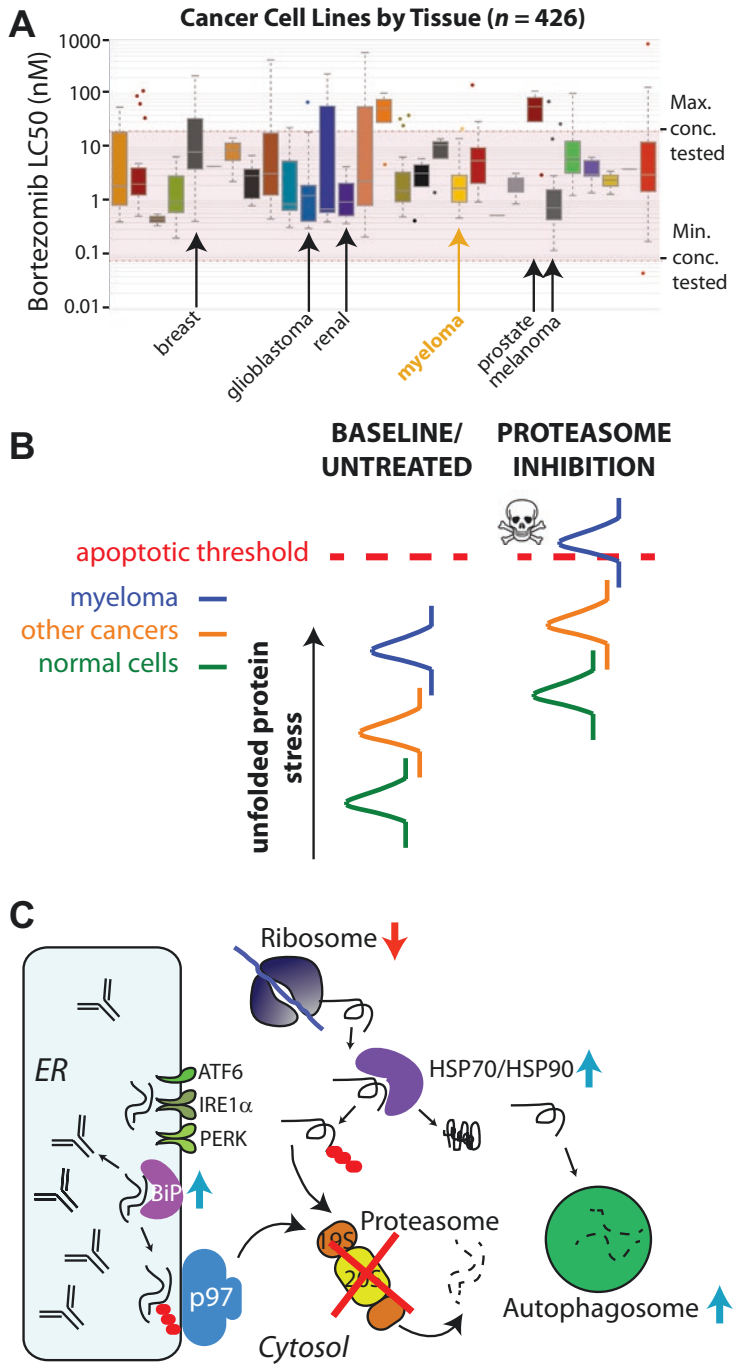

Fig. 10.2 Proteotoxicity-based mechanism of action of PIs and overcoming PI resistance by targeting protein homeostasis. (**a**). Screening of bortezomib vs. 426 cell lines in the Genomics of Drug Sensitivity in Cancer Database (www.cancerxgene.org). Sensitivity of specific tumor cell lines highlighted in comparison to myeloma lines. Boxes represent 2nd and 3rd quartile range, with whiskers to 1.5 times inter-quartile range (log-transformed). (**b**). Model describing selective *in vivo* sensitivity of multiple myeloma to proteasome inhibition, as a function of proteotoxic stress and unfolded protein response. (**c**). Alterations in protein homeostasis as a function of proteasome inhibition. With proteasomeal blockade (red "X"), there is a strong upregulation (blue arrows) in both cytosolic and endoplasmic reticulum chaperones as well as the autophagy system, while protein synthesis is decreased. Inability to decrease proteotoxicity via the proteasome may increase dependence on these other arms of the protein homeostasis response for myeloma plasma cell survival, revealing selective vulnerabilities in proteasome inhibitor-resistant plasma cells

adapted for propagation in a dish, often for decades, by picking up numerous genomic and phenotypic alterations from the original tumor (Ben-David et al. 2018; Liu et al. 2019b). In the case of MM cell lines this caveat is particularly relevant. First, these cell lines have almost all been derived from patients with very aggressive forms of MM that were able to circulate at high levels in the peripheral blood (Drexler and Matsuo 2000). Therefore, these lines do not well-represent the typical disease state of MM, which is highly dependent on the bone marrow microenvironment for survival (Bianchi and Munshi 2015; Manier et al. 2016). As part of this adaptation, many MM cell lines have also greatly decreased, or even eliminated, immunoglobulin secretion compared to primary MM tumor cells.

We consider these to be important observations in determining the predominant mechanism of action of PIs. First, PIs appear to have numerous effects on MM plasma cell adhesion and response to cytokines from the tumor microenvironment (Hideshima et al. 2011). Furthermore, PIs may influence the behavior of neighboring immune cells to further drive tumor cell death (Shanker et al. 2015). Therefore, PIs could conceivably disrupt malignant plasma cell-specific interactions given their unique microenvironment dependencies.

A second unique feature of MM plasma cells is related to high levels of protein synthesis involving the endoplasmic reticulum (ER). Originally proposed by Obeng et al. (Obeng et al. 2006), their studies showed that treatment of MM plasma cells with bortezomib led to activation of the unfolded protein response (UPR). The UPR is a complex set of cellular responses triggered by sensing of unfolded protein load in the ER (Walter and Ron 2011). Notably, activation of the UPR has dual functions. Initial responses triggered by activation of the kinase PERK, proteolytic cleavage and nuclear translocation of the transcription factor ATF6, and activation of the IRE1 ribonuclease lead to cellular adaptation to unfolded protein stress by, for example, attenuating translation, activating autophagy, and overexpression of protein-folding chaperones (Hetz and Papa 2018). This activation of the UPR is a normal and essential part of plasma cell development to accommodate physiological immunoglobulin synthesis (Gass et al. 2002; Shaffer et al. 2004). However, under prolonged unfolded protein stress, in excess of the cell's adaptive capacity, the PERK/ATF4- and IRE1/XBP1-mediated branches of the UPR can ultimately drive the synthesis of the transcription factor CHOP, which then leads to upregulation of the pro-apoptotic BH3-family protein NOXA (Iurlaro and Munoz-Pinedo 2016). In this way, the UPR governs a fine balance in the plasma cell by both mediating adaptation to proteotoxic stress but also triggering cell death when the stress is too great.

Therefore, one attractive model, though still unproven, to explain the relative sensitivity of MM plasma cells to proteasome inhibition is shown in Fig. 10.2b. At baseline, normal cells have very low amounts of proteotoxic stress and thereby have a large capacity to adapt to the additional stress induced by PIs before reaching the apoptotic threshold. Studies in neurons, for example, have suggested that at baseline only 20% of proteasomes are in a substrate-engaged state (Asano et al. 2015), suggesting that pharmacological inhibition of the majority of proteasomes could be tolerated in these cells. Other types of cancers have increased proteotoxic stress compared to normal cells, but still have significant dynamic range to adapt before they die. MM plasma cells, on the other hand, are already close to death due their baseline high levels of proteotoxic stress caused by unfolded immunoglobulin molecules. PIs can thereby push them over the threshold to cell death.

10.6 Resistance to Proteasome Inhibitors: A Complex and Common Problem in Myeloma

As noted above, clinical responses to PIs have been remarkable when compared to earlier MM therapies. However, the unfortunate reality is that PIs are not curative. Essentially every initially-responding patient treated with these drugs will

become resistant to them at some point ("acquired" resistance). Furthermore, many MM patients demonstrate "intrinsic" resistance to PIs, where they receive little clinical benefit from drug treatment but are still susceptible to toxic side effects.

In terms of acquired resistance to PIs, the answer at first appeared straightforward. Long-term incubation of MM cell lines with non-lethal doses of bortezomib resulted in resistant clones harboring mutations in *PSMB5*, which encodes the 20S β5 subunit targeted by the drug (Franke et al. 2016; Oerlemans et al. 2008). This concept was appealing as it drew analogies to kinase inhibitors such as the BCR-ABL inhibitor imatinib or the EGFR inhibitor erlotinib (Lovly and Shaw 2014), where single point mutations in the drug target drives resistance in patients. In MM, however, the story was not that simple. Only a handful of relapsed patients harboring *PSMB5* mutations have ever been identified (Barrio et al. 2019) despite widespread clinical resistance to bortezomib.

Therefore, alternate mechanisms of resistance had to be explored. Early studies using gene expression profiling in response to bortezomib provided initial hints. This work revealed induction of Nrf-1 activity to increase synthesis of proteasomal subunits, a very prominent HSF1-mediated heat shock response, and strong stimulation of autophagy after bortezomib treatment (Mitsiades et al. 2002). We found similar results by acquiring integrated transcriptomic and proteomic data after bortezomib treatment (Wiita et al. 2013). Together, these acute responses suggest possible mechanisms of long-term resistance, united by the effort to maintain protein homeostasis in the face of proteasomal blockade (Fig. 10.2c). These hypotheses were reinforced by proteomic analysis of *in vitro*-evolved resistant cell lines that did not harbor *PSMB5* mutations (Soriano et al. 2016). Comparison to parental cell line counterparts revealed prominent upregulation of both proteasome subunits and numerous protein-folding chaperones including multiple HSP70 and HSP90 isoforms and related co-chaperones (Soriano et al. 2016). However, we do note that many of these acquired alterations have not been definitively demonstrated to occur in MM patients, leaving their clinical importance a matter of uncertainty.

One compelling study suggested another potential path to resistance (Leung-Hagesteijn et al. 2013). In this work, Leung-Hagesteign et al. used gene expression signatures to show that expression levels of *XBP1* and *IRE1/ERN1* – prominent genes in the UPR pathway, that also help define the differentiation of plasma cells from B-cells (Carrasco et al. 2007) – were surprisingly lower in patients non-responsive to bortezomib. Indeed, careful analysis showed that patient tumor cells resistant to bortezomib had lost their plasma cell morphology and immunophenotype, reverting to looking much more like pre-plasmablasts (earlier plasma cell precursors) or B-cells (Leung-Hagesteijn et al. 2013). Parallel studies also confirmed that low XBP1 plasma cells were more resistant to bortezomib (Ling et al. 2012). This finding of resistance via de-differentation is intriguing for many reasons. First, the "cell of origin" for MM, where initial genomic lesions occur that ultimately drive disease, is thought to be a B-cell within the lymph node germinal center (Barwick et al. 2019). This de-differentiation may be part of a phenomenon of reversion to this precursor state, perhaps carrying a relationship to the elusive MM "stem cell". Second, this central finding, that plasma cell de-differentiation significantly decreases sensitivity to bortezomib, strongly supports the hypothesis that inducing high levels of unfolded protein stress in already-stressed plasma cells is the primary mechanism of action of PIs. Notably, deep surface immunophenotyping by flow cytometry of residual plasma cells in patient bone marrow after MM induction therapy (including bortezomib but also other agents) further suggests that plasma cells that survive this initial treatment are indeed different from the bulk tumor (Paiva et al. 2016).

In terms of intrinsic resistance to PIs, many important hints came from analysis of gene expression data in the APEX phase III trial of single agent bortezomib in MM (Richardson et al. 2005; Mulligan et al. 2007). In this study,

CD138+ tumor cells were isolated prior to treatment and submitted for gene expression profiling by microarray. Gene expression signatures at baseline could then be compared to subsequent clinical response. For example, one of the most surprising and prominent differences between responders and non-responders was expression of the gene *TJP1*, encoding the surface adhesion molecule tight junction protein 1. Mechanistic studies by Zhang et al. (Zhang et al. 2016) revealed that *TJP1* alters expression of immunoproteasome subunits and thereby regulates response to PI.

Shotgun proteomic profiling of patient plasma cells at baseline and subsequent correlation with response after combined bortezomib, doxorubicin, and dexamethasone therapy suggested that patients with poor response expressed higher levels of proteasome subunits, heat shock-related chaperones, and proteins related to mitigating reactive oxygen stress (Dytfeld et al. 2016).

In another finding with implications for intrinsic resistance, simultaneous papers from Tsvetkov et al. (Tsvetkov et al. 2015) and Acosta-Alvear et al. (Acosta-Alvear et al. 2015) both used genomic screening approaches to show that knockdown of several 19S subunits led to PI resistance. This finding was surprising given that prior to this work, it would be expected that genetic inhibition of any part of proteasome function would synergize with pharmacologic proteasome inhibition (indeed, this synergy was observed for depletion of 20S core subunits (Acosta-Alvear et al. 2015)). Notably, this finding was supported by patient data, where decreased expression of 19S lid subunits was found in carfilzomib non-responding vs. responding tumor cells (Acosta-Alvear et al. 2015). This observation has been further extended by Tsvetkov, Lindquist and colleagues, identifying correlations between 19S cap subunit expression and proteasome inhibitor sensitivity across all types of tumor cell lines (Tsvetkov et al. 2017). A very recent study, using stable inhibition of the 19S cap as a model system, also suggested that mitochondrial metabolism may play an important part in defining intrinsic resistance to PIs (Tsvetkov et al. 2019).

A thorough study by Mitra et al. (Mitra et al. 2017) profiled sensitivity to four different PIs (bortezomib, carfilzomib, oprozomib, mirazomib) across 50 MM cell lines. By RNA-seq analysis and machine learning approaches they identified a 42-gene signature defining intrinsic resistance that was also predictive of response to PI-including therapy in multiple clinical trials. While these genes were not strongly overrepresented by a single biological pathway, multiple proteasome subunit-encoding genes were included as well as HSP70 (Mitra et al. 2017).

Others have suggested that microenvironment effects play an important role in determining intrinsic resistance. Increased signaling in response to cytokines (such as IL-6, TGFβ, IGF-1) in the marrow microenvironment, or increased adhesion to stromal cells (mediated by surface proteins such as CXCR4), may also lead to intrinsic resistance to PIs (Di Marzo et al. 2016).

Taken together, extensive research into PI resistance has revealed a complex landscape of potential mechanisms. Furthermore, the large majority of studies have focused on bortezomib given that this is the agent with the most clinical experience. Differential resistance mechanisms between different PIs occurring in patients have not been well-defined. While it is likely that the broad scope of both mechanism of action and modes of resistance are similar in patients, it is likely that there are also at least some effects unique to each molecule. As described above, to some degree these differential effects are best-exemplified by prominent toxicities in patients – peripheral neuropathy in bortezomib and cardiotoxicity in carfilzomib – and by clinical response to carfilzomib after bortezomib resistance. As another example, in the evolved-resistance cell line study of Soriano et al. (Soriano et al. 2016), prominent upregulation of ABCB1 (also known as MDR1 or P-gp), a multi-drug efflux pump, was only found in carfilzomib-resistant plasma cells but not bortezomib-resistant. Follow up studies demonstrated that

ABCB1 was indeed upregulated in carfilzomib- but not bortezomib-resistant patient samples (Besse et al. 2018). Additional work is required to define factors leading to both differential acquired and intrinsic resistance for PIs beyond bortezomib.

10.7 Extending the Reach of Proteasome Inhibitors: Overcoming Resistance and Moving Beyond Myeloma

The studies outlined above, defining resistance mechanisms to PIs, are also important as they frequently suggest therapeutic targets by which to overcome PI resistance. Notably, many of these involve targeting other nodes in the cellular protein homeostasis network. The major hypothesis in this context is that if the proteasome is unavailable as a way to maintain cellular homeostasis, other mechanisms will compensate for this loss. However, this compensation creates new selective vulnerabilities in PI-resistant cells (Fig. 10.2c).

For instance, promising approaches to overcome bortezomib resistance, at least *in vitro*, include targeting de-ubiquitinases required for proteasomal function including USP7, USP14, and UCHL5 (Chauhan et al. 2012; Tian et al. 2014; Gavory et al. 2018). Similarly, we showed that pharmacologically inhibiting VCP/p97, a AAA+ ATPase responsible for translocating unfolded proteins from the ER to the proteasome for degradation, was able to overcome evolved PI resistance (Le Moigne et al. 2017). Our recent work with selective allosteric HSP70 inhibitors (Li et al. 2015; Shao et al. 2018) indicate that PI-resistant cell lines are indeed more sensitive to these inhibitors than their PI-sensitive counterparts (Ferguson et al. 2018). Together, these approaches will ideally reveal ways to either selectively target PI-resistant disease or design new combinations with PIs to extend efficacy of these remarkable agents.

As described above, these studies in MM strongly suggest that the primary mechanism of action of PIs relates to the exquisite sensitivity of plasma cells to perturbation of protein homeostasis. Therefore, the major question is whether there is any hope for PIs to be extended to other malignancies, and, in particular, solid tumors. We believe there are a few potential avenues to address this question, which are all currently being explored in clinical trials. One is the increased investigation of irreversible PIs such as carfilzomib or oprozomib. Irreversible inhibition of the proteasome, perhaps aided by dual inhibition of both β5 and β2 (Besse et al. 2019), may lead to deeper and more prolonged inhibition of the proteasome in solid tumors, allowing for crossing the threshold to apoptosis while still sparing normal cells (Fig. 10.2b). While initial clinical studies of advanced solid tumors treated with carfilzomib showed little evidence of response (Papadopoulos et al. 2013), some trials in solid tumors are still ongoing. Another parallel approach is taking advantage of *in vitro* cell line sensitivity data. Pharmacologic data revealed renal cell carcinoma cell lines to be among the most sensitive to PI treatment (Fig. 10.2a), and indeed a pilot trial of carfilzomib in RCC is currently underway (NCT01775930). Of course the caveats mentioned above, contrasting phenotypes of cell lines vs. tumors in patients, all still apply, but this clinical result will be intriguing to see nonetheless. Finally, one biology-driven strategy could be focusing on other tumor types known to have extremely high levels of protein secretion. In this case, pancreatic neuroendocrine tumors (PNETs), while rare, are particularly intriguing. These malignancies also exhibit extremely expanded ER networks, reminiscent of plasma cells, and are thought to be under high levels of baseline proteotoxic stress (Moore et al. 2019). A clinical trial is currently underway investigating carfilzomib in PNET and related neuroendocrine tumors (NCT02318784). The results of these trials will reveal whether PIs will be confined to their current indications or if they will finally be

able to expand their reach and benefit even more patients.

10.8 Lessons for More Broadly Targeting Protein Homeostasis in Cancer

The success of PIs in MM has led this malignancy to be the paradigm indication for any drug targeting protein homeostasis. Therefore it is worth considering the lessons learned from this experience.

- First, targeting protein homeostasis in any context is likely to lead to a broad array of potential mechanisms of action and potential mechanisms of resistance. For instance, in the case of chaperone inhibition: is there a specific, critical client that is degraded and leads to cell death? Or is death the result of cumulative effects across multiple different biological pathways?
- Second, therapeutic index is critical. Many of the protein homeostasis targets beyond the proteasome mentioned here (i.e. chaperones, p97, DUBs) are essential for cellular survival. PIs overcame initial skepticism to blaze the trail in this area, showing that you can interfere with these essential pathways and still lead to tumor cell death while sparing normal tissues. But the experience with PIs also suggests the biology has to be right: Is there a strong, preferential mechanism for tumor cell death vs. normal?
- Third, cell line studies are only partially predictive at best. The unique biology of tumors *in vivo* appears to have significant impact on the mechanism of action of proteostasis inhibitors. Therefore, it is important to use models (i.e. patient-derived xenografts, genetically-engineered mouse models, primary patient samples *ex vivo*, microenvironment-recapitulating culture systems) that do the best job of mimicking the tumor in patients when evaluating new small molecule inhibitors of protein homeostasis.

The success of PIs in MM has shown the promise of targeting protein homeostasis in cancer and established the paradigm test bed for novel agents. We look forward to ongoing efforts, using both existing molecules and new ones still to be developed, to target other protein homeostasis nodes across a broad range of malignancies. We certainly hope that some of these agents will one day join PIs in advancing from the bench to routine use in the clinic.

Acknowledgements A.P.W. would like to acknowledge research support from the Multiple Myeloma Research Foundation, Gabrielle's Angels Foundation for Cancer Research, Helen Diller Family Comprehensive Cancer Center at UCSF, and NIH grants K08 CA184116 and R01 CA226851 for supporting related work in his laboratory.

Disclosures A.P.W. is a member of the scientific advisory board and equity holder in Indapta Therapeutics and Protocol Intelligence. A.P.W. has received past research funding from TeneoBio, Sutro BioPharma, and Quadriga Biosciences.

References

Acosta-Alvear D et al (2015) Paradoxical resistance of multiple myeloma to proteasome inhibitors by decreased levels of 19S proteasomal subunits. elife 4:e08153

Adams J (2002) Development of the proteasome inhibitor PS-341. Oncologist 7(1):9–16

Adams J et al (1999) Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 59(11):2615–2622

Asano S et al (2015) Proteasomes. A molecular census of 26S proteasomes in intact neurons. Science 347(6220):439–442

Barrio S et al (2019) Spectrum and functional validation of PSMB5 mutations in multiple myeloma. Leukemia 33(2):447–456

Barwick BG, Gupta VA, Vertino PM, Boise LH (2019) Cell of origin and genetic alterations in the pathogenesis of multiple myeloma. Front Immunol. ePub ahead of print

Baugh JM, Viktorova EG, Pilipenko EV (2009) Proteasomes can degrade a significant proportion of cellular proteins independent of ubiquitination. J Mol Biol 386(3):814–827

Ben-David U et al (2018) Genetic and transcriptional evolution alters cancer cell line drug response. Nature 560(7718):325–330

Besse A et al (2018) Carfilzomib resistance due to ABCB1/MDR1 overexpression is overcome by nel-

finavir and lopinavir in multiple myeloma. Leukemia 32(2):391–401
Besse A et al (2019) Proteasome inhibition in multiple myeloma: head-to-head comparison of currently available proteasome inhibitors. Cell Chem Biol 26(3):340–351. e343
Bianchi G, Munshi NC (2015) Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood 125(20):3049–3058
Bianchi G et al (2009) The proteasome load versus capacity balance determines apoptotic sensitivity of multiple myeloma cells to proteasome inhibition. Blood 113(13):3040–3049
Boes B et al (1994) Interferon gamma stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes. J Exp Med 179(3):901–909
Carrasco DR et al (2007) The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell 11(4):349–360
Cenci S et al (2012) Pivotal advance: protein synthesis modulates responsiveness of differentiating and malignant plasma cells to proteasome inhibitors. J Leukoc Biol 92(5):921–931
Chatterjee S, Burns TF (2017) Targeting heat shock proteins in Cancer: a promising therapeutic approach. Int J Mol Sci 18(9):1978
Chauhan D et al (2005) A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell 8(5):407–419
Chauhan D et al (2011) In vitro and in vivo selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res 17(16):5311–5321
Chauhan D et al (2012) A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell 22(3):345–358
Choudhry P, Galligan D, Wiita AP (2018) Seeking convergence and cure with new myeloma therapies. Trends Cancer 4(8):567–582
Cromm PM, Crews CM (2017) The proteasome in modern drug discovery: second life of a highly valuable drug target. ACS Cent Sci 3(8):830–838
Cvek B, Dvorak Z (2011) The ubiquitin-proteasome system (UPS) and the mechanism of action of bortezomib. Curr Pharm Des 17(15):1483–1499
de Bruin G et al (2016) A set of activity-based probes to visualize human (Immuno)proteasome activities. Angew Chem 55(13):4199–4203
Demo SD et al (2007) Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res 67(13):6383–6391
Di Marzo L et al (2016) Microenvironment drug resistance in multiple myeloma: emerging new players. Oncotarget 7(37):60698–60711
Dimopoulos MA et al (2015) Retrospective matched-pairs analysis of bortezomib plus dexamethasone versus bortezomib monotherapy in relapsed multiple myeloma. Haematologica 100(1):100–106
Dimopoulos MA et al (2016) Carfilzomib and dexamethasone versus bortezomib and dexamethasone for patients with relapsed or refractory multiple myeloma (ENDEAVOR): a randomised, phase 3, open-label, multicentre study. Lancet Oncol 17(1):27–38
Dimopoulos MA et al (2017a) Carfilzomib or bortezomib in relapsed or refractory multiple myeloma (ENDEAVOR): an interim overall survival analysis of an open-label, randomised, phase 3 trial. Lancet Oncol 18(10):1327–1337
Dimopoulos MA et al (2017b) Cardiac and renal complications of carfilzomib in patients with multiple myeloma. Blood Adv 1(7):449–454
Dong Y et al (2019) Cryo-EM structures and dynamics of substrate-engaged human 26S proteasome. Nature 565(7737):49–55
Drexler HG, Matsuo Y (2000) Malignant hematopoietic cell lines: in vitro models for the study of multiple myeloma and plasma cell leukemia. Leuk Res 24(8):681–703
Durie BG et al (2017) Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. Lancet 389(10068):519–527
Dytfeld D et al (2016) Comparative proteomic profiling of refractory/relapsed multiple myeloma reveals biomarkers involved in resistance to bortezomib-based therapy. Oncotarget 7(35):56726–56736
Erales J, Coffino P (2014) Ubiquitin-independent proteasomal degradation. Biochim Biophys Acta 1843(1):216–221
Ettari R, Previti S, Bitto A, Grasso S, Zappala M (2016) Immunoproteasome-selective inhibitors: a promising strategy to treat hematologic malignancies, autoimmune and inflammatory diseases. Curr Med Chem 23(12):1217–1238
Fennell DA, Chacko A, Mutti L (2008) BCL-2 family regulation by the 20S proteasome inhibitor bortezomib. Oncogene 27(9):1189–1197
Ferguson I et al (2018) Novel allosteric inhibitors of heat shock protein 70 as agents to probe protein homeostasis and overcome proteasome inhibitor resistance in multiple myeloma. Blood (ASH Abstract) 132:3212
Fisher RI et al (2006) Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. J Clin Oncol 24(30):4867–4874
Franke M et al (2016) Formation of new chromatin domains determines pathogenicity of genomic duplications. Nature 538(7624):265–269
Gandolfi S et al (2017) The proteasome and proteasome inhibitors in multiple myeloma. Cancer Metastasis Rev 36(4):561–584
Gass JN, Gifford NM, Brewer JW (2002) Activation of an unfolded protein response during differentiation of antibody-secreting B cells. J Biol Chem 277(50):49047–49054

Gavory G et al (2018) Discovery and characterization of highly potent and selective allosteric USP7 inhibitors. Nat Chem Biol 14(2):118–125

Goldberg AL (2012) Development of proteasome inhibitors as research tools and cancer drugs. J Cell Biol 199(4):583–588

Harper JW, Bennett EJ (2016) Proteome complexity and the forces that drive proteome imbalance. Nature 537(7620):328–338

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479

Hetz C, Papa FR (2018) The unfolded protein response and cell fate control. Mol Cell 69(2):169–181

Hideshima T et al (2001) The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 61(7):3071–3076

Hideshima T et al (2002) NF-kappa B as a therapeutic target in multiple myeloma. J Biol Chem 277(19):16639–16647

Hideshima T, Richardson PG, Anderson KC (2011) Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. Mol Cancer Ther 10(11):2034–2042

Huang Z et al (2014) Efficacy of therapy with bortezomib in solid tumors: a review based on 32 clinical trials. Future Oncol 10(10):1795–1807

Huang X, Luan B, Wu J, Shi Y (2016) An atomic structure of the human 26S proteasome. Nat Struct Mol Biol 23(9):778–785

Huang HH et al (2020) Proteasome inhibitor-induced modulation reveals the spliceosome as a specific therapeutic vulnerability in multiple myeloma. Nat Commun. In press

Iurlaro R, Munoz-Pinedo C (2016) Cell death induced by endoplasmic reticulum stress. FEBS J 283(14):2640–2652

Jagannath S et al (2004) A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 127(2):165–172

Jariel-Encontre I, Bossis G, Piechaczyk M (2008) Ubiquitin-independent degradation of proteins by the proteasome. Biochim Biophys Acta 1786(2):153–177

Kenific CM, Debnath J (2015) Cellular and metabolic functions for autophagy in cancer cells. Trends Cell Biol 25(1):37–45

Kish-Trier E, Hill CP (2013) Structural biology of the proteasome. Annu Rev Biophys 42:29–49

Kleiger G, Mayor T (2014) Perilous journey: a tour of the ubiquitin-proteasome system. Trends Cell Biol 24(6):352–359

Kraus M et al (2015) The novel beta2-selective proteasome inhibitor LU-102 synergizes with bortezomib and carfilzomib to overcome proteasome inhibitor resistance of myeloma cells. Haematologica 100(10):1350–1360

Kuhn DJ et al (2007) Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood 110(9):3281–3290

Kupperman E et al (2010) Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. Cancer Res 70(5):1970–1980

Lander GC et al (2012) Complete subunit architecture of the proteasome regulatory particle. Nature 482(7384):186–191

Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149(2):274–293

Lasker K et al (2012) Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. Proc Natl Acad Sci U S A 109(5):1380–1387

Le Moigne R et al (2017) The p97 inhibitor CB-5083 is a unique disrupter of protein homeostasis in models of Multiple Myeloma. Mol Cancer Ther 16(11):2375–2386

Leleu X et al (2019) Role of proteasome inhibitors in relapsed and/or refractory multiple myeloma. Clin Lymphoma Myeloma Leuk 19(1):9–22

Leung-Hagesteijn C et al (2013) Xbp1s-negative tumor B cells and pre-plasmablasts mediate therapeutic proteasome inhibitor resistance in multiple myeloma. Cancer Cell 24(3):289–304

Levy JMM, Towers CG, Thorburn A (2017) Targeting autophagy in cancer. Nat Rev Cancer 17(9):528–542

Li C, Li R, Grandis JR, Johnson DE (2008) Bortezomib induces apoptosis via Bim and Bik up-regulation and synergizes with cisplatin in the killing of head and neck squamous cell carcinoma cells. Mol Cancer Ther 7(6):1647–1655

Li X et al (2015) Validation of the Hsp70-Bag3 protein-protein interaction as a potential therapeutic target in cancer. Mol Cancer Ther 14(3):642–648

Ling SC et al (2012) Response of myeloma to the proteasome inhibitor bortezomib is correlated with the unfolded protein response regulator XBP-1. Haematologica 97(1):64–72

Liu T, Zhou L, Li D, Andl T, Zhang Y (2019a) Cancer-associated fibroblasts build and secure the tumor microenvironment. Front Cell Dev Biol 7:60

Liu Y et al (2019b) Multi-omic measurements of heterogeneity in HeLa cells across laboratories. Nat Biotechnol 37(3):314–322

Lovly CM, Shaw AT (2014) Molecular pathways: resistance to kinase inhibitors and implications for therapeutic strategies. Clin Cancer Res 20(9):2249–2256

Manier S, Kawano Y, Bianchi G, Roccaro AM, Ghobrial IM (2016) Cell autonomous and microenvironmental regulation of tumor progression in precursor states of multiple myeloma. Curr Opin Hematol 23(4):426–433

Matthews GM et al (2016) NF-kappaB dysregulation in multiple myeloma. Semin Cancer Biol 39:68–76

Mitra AK et al (2017) A gene expression signature distinguishes innate response and resistance to proteasome inhibitors in multiple myeloma. Blood Cancer J 7(6):e581

Mitsiades N et al (2002) Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A 99(22):14374–14379

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147(4):728–741

Moore PC et al (2019) Parallel signaling through IRE1α and PERK regulates pancreatic neuroendocrine tumor growth and survival. Cancer Res 79(24):6190–6203

Mulligan G et al (2007) Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood 109(8):3177–3188

Nussbaum AK et al (1998) Cleavage motifs of the yeast 20S proteasome beta subunits deduced from digests of enolase 1. Proc Natl Acad Sci U S A 95(21):12504–12509

O'Connor OA et al (2009) A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. Clin Cancer Res 15(22):7085–7091

Obeng EA et al (2006) Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood 107(12):4907–4916

Oerlemans R et al (2008) Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. Blood 112(6):2489–2499

Paiva B et al (2016) Phenotypic and genomic analysis of multiple myeloma minimal residual disease tumor cells: a new model to understand chemoresistance. Blood 127(15):1896–1906

Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 78(5):773–785

Papadopoulos KP et al (2013) A phase I/II study of carfilzomib 2-10-min infusion in patients with advanced solid tumors. Cancer Chemother Pharmacol 72(4):861–868

Peters JM, Cejka Z, Harris JR, Kleinschmidt JA, Baumeister W (1993) Structural features of the 26 S proteasome complex. J Mol Biol 234(4):932–937

Petrucci MT, Finsinger P, Chisini M, Gentilini F (2014) Subcutaneous bortezomib for multiple myeloma treatment: patients' benefits. Patient Prefer Adherence 8:939–946

Ravid T, Hochstrasser M (2008) Diversity of degradation signals in the ubiquitin-proteasome system. Nat Rev Mol Cell Biol 9(9):679–690

Rechsteiner M, Hill CP (2005) Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors. Trends Cell Biol 15(1):27–33

Reece DE et al (2009) Weekly and twice-weekly bortezomib in patients with systemic AL amyloidosis: results of a phase 1 dose-escalation study. Blood 114(8):1489–1497

Richardson PG et al (2003) A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 348(26):2609–2617

Richardson PG et al (2005) Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 352(24):2487–2498

Roeten MSF, Cloos J, Jansen G (2018) Positioning of proteasome inhibitors in therapy of solid malignancies. Cancer Chemother Pharmacol 81(2):227–243

San Miguel JF et al (2008) Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 359(9):906–917

Saric T, Graef CI, Goldberg AL (2004) Pathway for degradation of peptides generated by proteasomes: a key role for thimet oligopeptidase and other metallopeptidases. J Biol Chem 279(45):46723–46732

Schubert U et al (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404(6779):770–774

Shaffer AL et al (2004) XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. Immunity 21(1):81–93

Shanker A, Dikov MM, Carbone DP (2015) Promise of immunotherapy in lung Cancer. Prog Tumor Res 42:95–109

Shao H et al (2018) Exploration of Benzothiazole Rhodacyanines as allosteric inhibitors of protein-protein interactions with heat shock protein 70 (Hsp70). J Med Chem 61(14):6163–6177

Siegel DS et al (2018) Improvement in overall survival with Carfilzomib, Lenalidomide, and dexamethasone in patients with relapsed or refractory multiple myeloma. J Clin Oncol 36(8):728–734

Sijts EJ, Kloetzel PM (2011) The role of the proteasome in the generation of MHC class I ligands and immune responses. Cell Mol Life Sci 68(9):1491–1502

Sledz P, Baumeister W (2016) Structure-driven developments of 26S proteasome inhibitors. Annu Rev Pharmacol Toxicol 56:191–209

Soriano GP et al (2016) Proteasome inhibitor-adapted myeloma cells are largely independent from proteasome activity and show complex proteomic changes, in particular in redox and energy metabolism. Leukemia *30*(11):2198

Spencer A et al (2018a) Daratumumab plus bortezomib and dexamethasone versus bortezomib and dexamethasone in relapsed or refractory multiple myeloma: updated analysis of CASTOR. Haematologica 103(12):2079–2087

Spencer A et al (2018b) A phase 1 clinical trial evaluating marizomib, pomalidomide and low-dose dexamethasone in relapsed and refractory multiple myeloma (NPI-0052-107): final study results. Br J Haematol 180(1):41–51

Stewart AK et al (2015) Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med 372(2):142–152

Strauss SJ et al (2007) The proteasome inhibitor bortezomib acts independently of p53 and induces cell death via apoptosis and mitotic catastrophe in B-cell lymphoma cell lines. Cancer Res 67(6):2783–2790

Suraweera A, Munch C, Hanssum A, Bertolotti A (2012) Failure of amino acid homeostasis causes cell death following proteasome inhibition. Mol Cell 48(2):242–253

Tanaka K (2009) The proteasome: overview of structure and functions. Proc Jpn Acad Ser B Phys Biol Sci 85(1):12–36

Tian Z et al (2014) A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. Blood 123(5):706–716

Traenckner EB, Wilk S, Baeuerle PA (1994) A proteasome inhibitor prevents activation of NF-kappa B and stabilizes a newly phosphorylated form of I kappa B-alpha that is still bound to NF-kappa B. EMBO J 13(22):5433–5441

Treon SP et al (2009) Primary therapy of Waldenstrom macroglobulinemia with bortezomib, dexamethasone, and rituximab: WMCTG clinical trial 05-180. J Clin Oncol 27(23):3830–3835

Tsherniak A et al (2017) Defining a Cancer dependency map. Cell 170(3):564–576. e516

Tsvetkov P et al (2015) Compromising the 19S proteasome complex protects cells from reduced flux through the proteasome. elife 4:e08467

Tsvetkov P et al (2017) Suppression of 19S proteasome subunits marks emergence of an altered cell state in diverse cancers. Proc Natl Acad Sci U S A 114(2):382–387

Tsvetkov P et al (2018) Oncogenic addiction to high 26S proteasome level. Cell Death Dis 9(7):773

Tsvetkov P et al (2019) Mitochondrial metabolism promotes adaptation to proteotoxic stress. Nat Chem Biol 15(7):681–689

Ustrell V, Pratt G, Rechsteiner M (1995) Effects of interferon gamma and major histocompatibility complex-encoded subunits on peptidase activities of human multicatalytic proteases. Proc Natl Acad Sci U S A 92(2):584–588

von Mikecz A (2006) The nuclear ubiquitin-proteasome system. J Cell Sci 119(Pt 10):1977–1984

Walter P, Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. Science 334(6059):1081–1086

Waxman AJ et al (2018) Carfilzomib-associated cardiovascular adverse events: a systematic review and meta-analysis. JAMA Oncol 4(3):e174519

Weyburne ES et al (2017) Inhibition of the proteasome beta2 site sensitizes triple-negative breast Cancer cells to beta5 inhibitors and suppresses Nrf1 activation. Cell Chem Biol 24(2):218–230

Wiita AP et al (2013) Global cellular response to chemotherapy-induced apoptosis. elife 2:e01236

Winter MB et al (2017) Immunoproteasome functions explained by divergence in cleavage specificity and regulation. elife 6:e27364

Wolfe AL et al (2014) RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. Nature 513(7516):65–70

Yoshimura T et al (1993) Molecular characterization of the “26S” proteasome complex from rat liver. J Struct Biol 111(3):200–211

Zhang XD et al (2016) Tight junction protein 1 modulates proteasome capacity and proteasome inhibitor sensitivity in multiple myeloma via EGFR/JAK1/STAT3 Signaling. Cancer Cell 29(5):639–652

Zhao J, Zhai B, Gygi SP, Goldberg AL (2015) mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy. Proc Natl Acad Sci U S A 112(52):15790–15797

11 Reflections and Outlook on Targeting HSP90, HSP70 and HSF1 in Cancer: A Personal Perspective

Paul Workman

Abstract

This personal perspective focuses on small-molecule inhibitors of proteostasis networks in cancer—specifically the discovery and development of chemical probes and drugs acting on the molecular chaperones HSP90 and HSP70, and on the HSF1 stress pathway. Emphasis is on progress made and lessons learned and a future outlook is provided. Highly potent, selective HSP90 inhibitors have proved invaluable in exploring the role of this molecular chaperone family in biology and disease pathology. Clinical activity was observed, especially in non small cell lung cancer and HER2 positive breast cancer. Optimal use of HSP90 inhibitors in oncology will likely require development of creative combination strategies. HSP70 family members have proved technically harder to drug. However, recent progress has been made towards useful chemical tool compounds and these may signpost future clinical drug candidates. The HSF1 stress pathway is strongly validated as a target for cancer therapy. HSF1 itself is a ligandless transcription factor that is extremely challenging to drug directly. HSF1 pathway inhibitors have been identified mostly by phenotypic screening, including a series of bisamides from which a clinical candidate has been identified for treatment of ovarian cancer, multiple myeloma and potentially other cancers.

Keywords

HSP90 · HSP70 · HSF1 · Chemical probes · Drug discovery · Biomarkers · Translational research

11.1 Introduction

It seems very fitting that a collection of articles on proteostasis networks in cancer – and one that is dedicated to the extraordinary life and work of Sue Lindquist – should conclude with a piece on experience with preclinical and clinical translation in this area. I feel privileged to be invited to provide a short article on my reflections on progress made, lessons learned and an outlook for the future in this field. This brief commentary is written very much as a personal perspective – based on more than two decades of involvement working on chemical biology and drug discovery

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_11

around HSP90, HSP70 and HSF1 as molecular targets. I will mainly illustrate the progress and challenges based on the work carried out in my own laboratory – in association with many talented colleagues and collaborators. I will also refer to the work of others, but I apologise to the many excellent scientists in the field that it is in the nature of a short personal perspective that exhaustive referencing is not possible.

Sue Lindquist was an outstanding scientist and true pioneer in the field of proteostasis. Her curiosity and great aptitude for the basic science coupled with a strong desire to make a difference to human disease led her to work and collaborate across many research boundaries. Such an approach is often critically important to make real breakthroughs in fundamental research and is certainly essential to translate basic research into clinical benefit. In this and in her contributions to the development of the field and the careers of scientists who work in it, Sue was an inspiration to us all.

11.2 Therapeutic Targeting of HSP90

My lab started working on the Heat Shock Protein (HSP) and molecular chaperone HSP90 in the mid-to-late 1990's as a serendipitous result of the convergence of the two main strands of our molecular pharmacology and drug discovery research at the time. The first strand was the design of drugs that would selectively kill cancer cells by virtue of their preferential bioreductive conversion to cytotoxic metabolites – either as a result of the hypoxic nature of solid tumours or/and the increased expression of enzymes that catalyse their bioreductive activation (Workman and Stratford 1993). The second strand was the design of drugs that exploit and counteract the oncogenic pathways involved in the maintenance and progression of cancer, now referred to as 'oncogene addiction' (Brunton and Workman 1993).

The coalescing of these two strands is illustrated by a study carried out in my lab to test two hypotheses to explain the molecular mode of action by which the natural product geldanamycin – a member of the benzoquinone ansamycin class of natural product antibiotic – exerts its nonclinical anticancer activity in human colorectal cancer cells. See Fig. 11.1 for the chemical structure of geldanamycin and other selected compounds and drugs referred to in this article. The two possibilities we tested were that the anticancer effect was mediated either through: (1) bioreductive activation of the quinone moiety catalysed by the flavoenzyme oxidoreductase NAD(P)H: quinone oxidoreductase (EC 1.6.99.2; known as NQO1 and previously as DT-diaphorase); or (2) depletion of the cellular c-SRC oncoprotein as a result of inhibition of the molecular chaperone HSP90. These two hypotheses did not appear from thin air. Exemplified by research on another natural product called mitomycin C, the bioreductive metabolic activation of quinone-group containing agents by NQO1 and other oxidoreductases to produce DNA-binding metabolites was well precedented as an approach to killing cancer cells through the work of the groups of Alan Sartorelli (Sartorelli 1988) and later David Ross (Ross et al. 2000) and others. Indeed, our own lab showed that cancer cell sensitivity to both mitomycin C and the synthetic indoloquinone EO9 is determined by the level of expression of NQO1 – which is often very high in some cancer cell lines that tend to be sensitive, or alternatively low or absent in others that tend to be resistant (Walton et al. 1992; Fitzsimmons et al. 1996). The alternative hypothesis that we tested was that the anticancer effect of geldanamycin and related agents such as herbimycin is a result of the inhibition of the activity of cellular SRC – a kinase that has been implicated in colorectal cancer initiation and progression (Cartwright et al. 1990; Garcia et al. 1991).

In our initial study we largely ruled out that geldanamycin kills human colorectal cancer cells through either bioreductive metabolism by NQO1 or inhibition of cellular c-SRC kinase activity (Brunton et al. 1998). Firstly, we showed that although NQO1 does reduce geldanamycin this could not explain its anticancer activity. And secondly, we observed no effect on cellular c-SRC at pharmacologically active concentrations. We did observe a decrease in c-SRC kinase activity in the colorectal cancer cells at very high concentrations and prolonged exposure to geldanamycin, but this is due to a reduction in

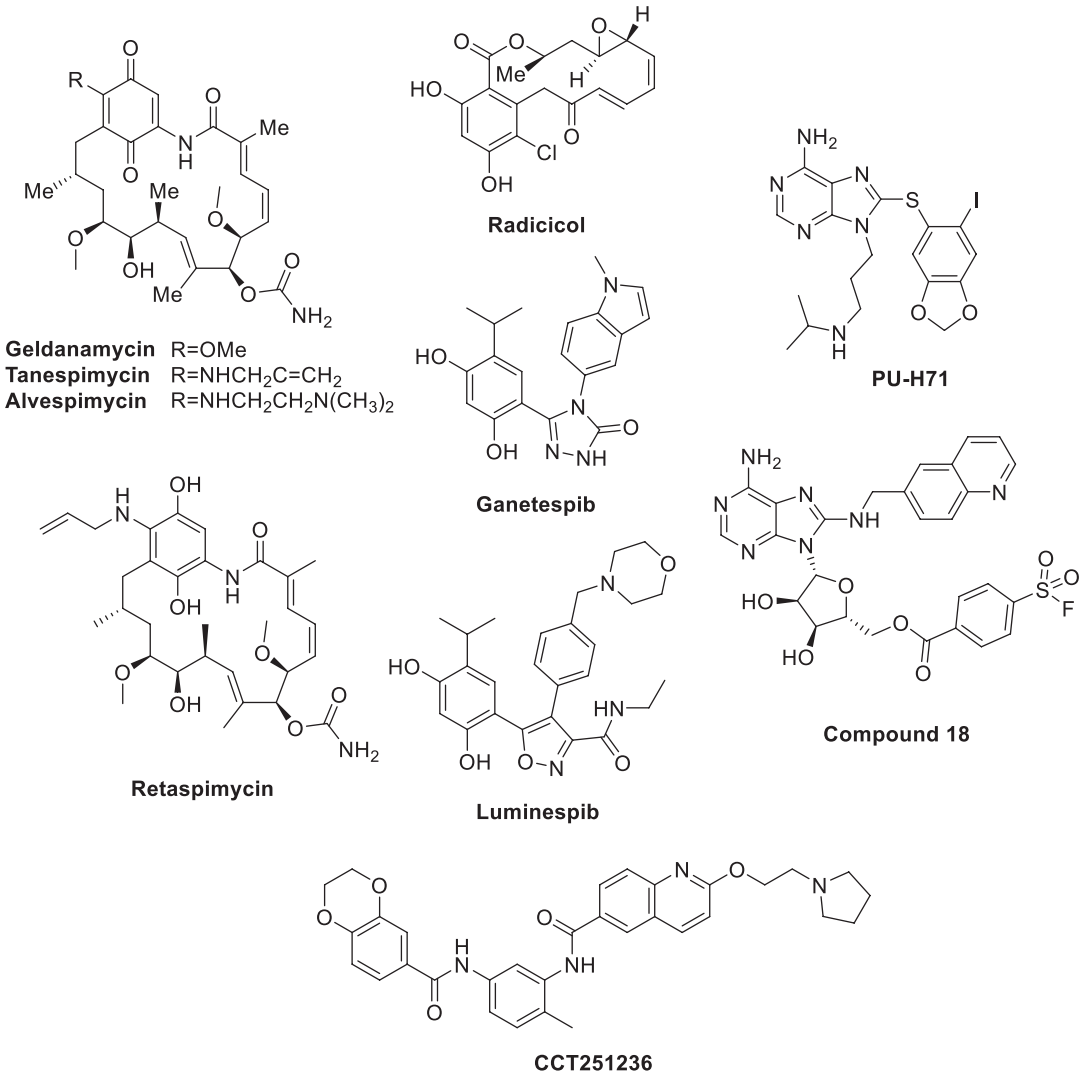

Fig. 11.1 Chemical structures of selected examples of compounds and drugs referred to in this article

overall c-SRC protein levels. Indeed, there had been previous reports of depletion of SRC and other tyrosine kinase oncoproteins in response to geldanamycin and herbimycin A. Furthermore, in what is now regarded as a seminal discovery in the field published by Luke Whitesell working in Len Neckers lab, it was shown that the major target to which geldanamycin is bound in the cell is a 90 kDa protein that was identified as the heat shock protein HSP90 – and moreover it was demonstrated that this led to the disruption of the known interaction between HSP90 and the oncogenic viral form of the tyrosine kinase v-SRC (Whitesell et al. 1994). These findings explained the previously demonstrated ability of geldanamycin to revert the oncogenic transformation of mammalian cells by viral SRC, rather than inhibiting SRC's intrinsic tyrosine kinase activity, and pointed to HSP90 as a potential target in cancer by bringing about the depletion of oncogenic client proteins by proteosomal degradation. Despite this interesting and attractive mechanism of action, geldanamycin did not progress to clinical studies due to concerns about liver toxicity.

However, semi-synthetic derivatives of geldanamycin subsequently showed more promise as drugs. Thus we later returned, with more translational success this time, to study both the impact of

bioreductive metabolism of the quinone moiety and also the depletion of oncogenic kinases and other 'client' proteins of the HSP90 molecular chaperone following exposure of cancer cells to the analogue of geldanamycin that was commonly (and still is) referred to as 17-AAG (17-allylamino-17-demethoxygeldanamycin) – subsequently named tanespimycin. This drug was progressed to the clinic because it maintained the anticancer effect of geldanamycin but was better tolerated in animals and has an acceptable therapeutic index. In collaboration with colleagues at the US National Cancer Institute (NCI) we showed that in contrast to geldanamycin – but similar to the bioreductive quinones mitomycin C and EO9 (see above) – there is a clear correlation between sensitivity to tanespimycin and NQO1 expression in the NCI collection of 60 human cancer cell lines (the so-called 'NCI 60' panel), with greater sensitivity seen in those lines with high NQO1 and lower sensitivity in those with low expression, including lines with a polymorphism resulting in reduced enzyme activity and stability (Kelland et al. 1999). We confirmed a causal relationship in an isogenic pair model either lacking NQO1 or exhibiting high expression. The strong dependence of cancer cell sensitivity on NQO1 of tanespimycin in contrast to geldanamycin was explained by our demonstration in the same paper that 17-AAG is a much better substrate for NQO1 than geldanamycin. Subsequent work by the Ross lab and others showed that the reduced hydroquinone form of tanespimycin is a more potent binder of HSP90 than the parent quinone 17-AAG itself (Guo et al. 2005).

I would like to make three observations about our discovery of NQO1 as a mechanism-based biomarker of tanespimycin sensitivity. The first point is that although we emphasized in several publications that NQO1 expression should be considered when comparing the sensitivity of different cancer cell lines to tanespimycin *in vitro*, this predictive biomarker relationship is generally ignored. Note, however, that although important in cell culture, NQO1 is likely to be less important in animal (and human) studies due to the metabolism of tanespimycin to 17-amino-geldanamycin – which we showed behaves like geldanamycin in being independent of NQO1 (Kelland et al. 1999). The second point is that the NQO1 effect is, of course, absent from non-quinone HSP90 inhibitors and not relevant for these (ref. (Kelland et al. 1999) and see later). The third point is that it was pleasing when our demonstration of NQO1 as a predictor of cancer cell sensitivity to tanespimycin – that we made using the NCI-60 cell panel – was subsequently confirmed as one of the strongest predictive genetic-pharmacology relationships in the first two major publications on the much bigger panels of hundreds of cancer cell lines that are now commonly used for such correlative analysis and predictive biomarker discovery (Barretina et al. 2012; Garnett et al. 2012). We discussed these findings in a commentary (Workman et al. 2012).

In our first above-mentioned publication on tanespimycin (Kelland et al. 1999) we demonstrated depletion of CRAF and mutant p53 as representative client proteins in cancer cells and the increased expression of HSP72 as a representative heat shock gene product that is upregulated in an HSF1-dependent manner following HSP90 inhibition. Following earlier work on geldanamycin and another natural product radicicol by others, we showed in human colon cancer models that tanespimycin inhibits oncogenic signal transduction in the MAP kinase and PI3 kinase pathways through depletion of HSP90 clients such as CRAF and AKT, resulting in cytostasis and apoptosis (Hostein et al. 2001). Similar findings were made by other labs, including the Neal Rosen group, showing for example that tanespimycin induces G1 cell cycle arrest, apoptosis and morphological and functional differentiation in breast cancer cells and cell cycle arrest and antitumour activity in prostate cancer models, both associated with depletion of the highly sensitive oncogenic HSP90 client protein HER2/ERBB2, steroid hormone receptors and other client proteins (Münster et al. 2001; Basso et al. 2002; Solit et al. 2002). Both the Rosen team and also the Richard Marais lab in collaboration with our group discovered independently that many mutant forms of BRAF, including the common V600E mutant that is the key oncogenic driver of melanoma and other cancers, are more highly

dependent on HSP90 and are more rapidly depleted by 17-AAG treatment (Grbovic et al. 2006; da Rocha Dias et al. 2005) – an effect subsequently seen with other mutant/wild type protein pairs.

Our lab was an earlier adopter of gene expression microarrays which we used to profile large-scale transcriptional changes in response to HSP90 inhibition (Clarke et al. 2000) and we also used mass spectrometry-based proteomics to profile broad changes in cellular proteins at scale (Maloney et al. 2007). Studies from many labs have detailed the depletion by proteosomal degradation of multiple oncogenic HSP90 client proteins following inhibition of the molecular chaperone. An online listing of the very large number of client proteins and other HSP90 interacting proteins is maintained by Didier Picard (HSP90 Interactors n.d.). A large-scale study from the Lindquist lab provided a systematic and quantitative survey – using the LUMIER assay system – of human kinases, transcription factors, and E3 ligases with respect to interaction with HSP90 and its cochaperone CDC37 (Taipale et al. 2012). This comprehensive analysis confirmed and enhanced our appreciation that many kinases interact with HSP90 but with different binding affinities – concluding (1) that kinase client binding specificity is dictated by CDC37 and (2) that the extent of depletion after HSP90 inhibition is greatest for strong binders and least for weak binders. A further conclusion was that the interaction of kinases with the HSP90/CDC37 system is dictated by the thermodynamic stability of their kinase folds. HSP90 client kinases are intrinsically less stable than non-client kinases. This finding helped us to understand why the ability to act as an HSP90 client protein cannot be predicted from the DNA or protein sequence. The study also revealed that relatively few transcription factors interact with HSP90, in contrast to the many E3 ligases and the majority of kinases (Taipale et al. 2012).

A number of protagonists supported the concept of progression of HSP90 inhibitors, including our own lab and those of Len Neckers, Neal Rosen, Ed Sausville, Gabriella Chiosis and Francis Burrows. For example, in ref. (Workman et al. 2007) a group of us reviewed the potential advantages of HSP90 inhibitors, especially the simultaneous and combinatorial degradation of multiple oncogenic client proteins, resulting in the blockade of multiple oncogenic pathways and antagonism of all of the pathological hallmark traits of malignancy – with cancer selectivity being achieved by exploiting cancer dependencies and vulnerabilities, including both oncogene addiction and the stressed state of malignant cells. We also argued that multiple downstream effects of HSP90 inhibitors should make the development of resistance more difficult compared to drugs exhibiting more limited effects. Of course the other side of the coin is the potential for significant toxicity, even though many of the affected proteins and pathways are more important to cancer compared to normal cells.

Working with our collaborators and supported by the US National Cancer Institute and what is now Cancer Research UK, it was exciting for us when we took tanespimycin into a first-in-human clinical trial in the Drug Development Unit of the Institute of Cancer Research and our hospital partner the Royal Marsden. To enable pharmacokinetic-pharmacodynamic (PK-PD) studies, we validated a protein biomarker signature of HSP90 inhibition, comprising depletion of HSP90 client proteins CRAF and CDK4 together with increased expression of HSP72 (Banerji et al. 2005a). CRAF and CDK were selected as biomarkers that are depleted, because of their applicability for measurement in peripheral blood lymphocytes (PBLs) and multiple cancers. In addition, HSP72 represents an ‘up’ biomarker that is robustly induced upon HSP90 inhibition across a wide range of biological contexts. It should be noted that HSP72 is a more sensitive biomarker of HSP90 inhibition, occurring at lower tanespimycin concentrations and doses; however, depletion of client proteins, which requires higher exposures, is more likely to be indicative of therapeutic effect. Evidence of HSP72 induction in the absence of client protein depletion is not sufficient to conclude that the degree of target modulation will have a reasonable chance of seeing a therapeutic effect. The combined biomarker signature of HSP72 induc-

tion and client protein depletion was used in our hypothesis-testing, PK-PK biomarker-led Phase I study of tanespimycin (Banerji et al. 2005b) that applied the Pharmacological Audit Trail (PhAT) framework that my colleagues and I developed for use in nonclinical and clinical studies to link target modulation to biological effects (Banerji and Workman 2016; Workman 2003). PK properties were consistent with target coverage and the PD biomarkers demonstrated HSP90 inhibition in PBLs and tissue biopsies. Our Phase I clinical study of tanespimycin provided proof-of-concept that HSP90 could be inhibited at doses that were tolerated by cancer patients. Dose-limiting side-effects were defined as diarrhoea and liver toxicity. Two patients with metastatic melanoma had stable disease for 15 and 49 months respectively; of note is that these patients' tumours had a V600E BRAF and a G13D NAS mutation, respectively, and thus both cancers would have contained active forms of the HSP90 client protein BRAF (Banerji et al. 2008).

Tanespimycin progressed to a large number of Phase II studies and showed its most promising activity in combination with the HER2 antibody trastuzumab in patients with HER2-positive metastatic breast cancer who were progressing on trastuzumab (Modi et al. 2011). The trial definitively showed objective responses according to Response Evaluation Criteria in Solid Tumors (RECIST) in these patients and the overall response rate was 22% with a clinical benefit rate of 59%. The effectiveness of tanespimycin in HER2-driven cancers makes mechanistic sense given that HER2 is one of the most highly sensitive HSP90 client proteins to HSP90 inhibition. However, despite its promise the clinical development of tanespimycin as a cancer therapy was terminated by the company for nonclinical, potentially commercial reasons such as costly production/formulation and patent expiry concerns (Arteaga 2011; Neckers and Workman 2012; Garcia-Carbonero et al. 2013). Subsequently, early clinical trials were carried out with additional geldanamycin analogues – specifically alvespimycin which is less dependent on NQO1 (see earlier) and the soluble stabilized hydroquinone form of tanespimycin, retaspimycin, but these have not progressed further. Factors that have been considered as limitations for tanespimycin and its analogues include insufficient depletion of key client proteins, the requirement for activation by NQO1, and side-effects – including the liver toxicity that may have been related to the quinone moiety underdoing bioreductive activation, as discussed above (see also ref. (Neckers and Workman 2012)).

Nevertheless, the proof of concept and promising activity in breast cancer shown by tanespimycin stimulated research to discover second generation, wholly synthetic, non-quinone inhibitors that might overcome the limitations of the geldanamycin class. Two particular new classes initially led the way, namely (1) the purine inhibitors based on PU-3 from Chiosis and colleagues at Memorial Sloan Kettering leading to the clinical candidate PU-H71 and others from Conforma/Biogen (Chiosis et al. 2001; Speranza et al. 2018) and (2) the resorcinol-type compounds exemplified by ganetespib from Synta Pharmaceuticals (Ying et al. 2012) and luminespib from ICR/Vernalis (Eccles et al. 2008; Sessa et al. 2013). Numerous other structurally diverse inhibitors were subsequently discovered and many progressed to the clinic (Travers et al. 2012).

Our own drug discovery research on HSP90 began with our identification by high-throughput screening at the ICR of the diaryl pyrazole resorcinol CCT018159 (Cheung et al. 2005; Sharp et al. 2007a). Improvements on this chemical starting point were enabled by the crystal structure of HSP90 solved by our collaborator Laurence Pearl and colleagues (Prodromou et al. 1997) which revealed precisely how CCT018159 is bound at the nucleotide site in the N-terminal domain of HSP90, mimicking the binding mode of the resorcinylic natural product radicicol (Roe et al. 1999). Our structure-based optimization based on the screening hit CCT018159 yielded more potent, advanced leads and chemical tools, specifically the potent resorcinylic pyrazole/isoxazole analogues VER-49009 and VER-50589 (Sharp et al. 2007b) and in turn resulted in our intravenously administered clinical candidate, the 4,5-diaryl isoxazole resorcinol luminespib

(Eccles et al. 2008; Brough et al. 2008). In addition, our collaboration between ICR and Vernalis yielded the oral back-up clinical candidate NVP-BEP800 from a 2-aminothieno[2,3-*d*]pyrimidine series, derived from a fragment-based and *in silico* hit-finding approach and optimized by structure-based design (Brough et al. 2009). Both series were licenced to Novartis.

Luminespib was the first of the second generation non-geldanamycin HSP90 inhibitors for which full results of a phase I study were reported (Sessa et al. 2013). In this Phase I clinical trial of intravenous luminespib led by Udai Banerji, the main side-effects were diarrhoea, asthenia/fatigue, anorexia, atrial flutter and visual symptoms and the recommended phase II dose (RP2D) was 70 mg/m^2. Application of the PhAT showed that the plasma concentrations of luminespib that were achieved were consistent with those that produced therapeutic effects in a range of human tumour xenograft models. In addition, evidence of target modulation was obtained in peripheral blood mononuclear cells (dose-dependent HSP72 induction) and tumour tissue (HSP70 induction and depletion of client protein AKT in two ER-positive patients at 70 mg/m^2). There were no radiological responses, which was attributed potentially to the fact that patients were not molecularly prioritized based on client protein status. However, dose-dependent metabolic responses were observed in a number of patients as measured by a reduction of ^{18}F-fluorodeoxyglucose uptake by PET scan. Based on these Phase I data, Phase II studies were initiated with the dose of 70 mg/m^2 in breast, gastric and non-small cell lung cancers with appropriate HSP90 client protein dependencies.

Evidence of activity of luminespib was observed in HER2-positive and ER-positive breast cancer (Schroder et al. 2011; Kong et al. 2016). A Phase II trial with luminespib in NSCLC included patient cohorts with EGFR-mutant, ALK-rearranged and KRAS-mutant disease (Felip et al. 2018). The overall response rate of 13% was modest and no objective responses were observed in patients with oncogenic KRAS mutation. In contrast, the most favourable outcome was seen in patients with oncogenic ALK rearrangement, for whom the objective response rate was 32%, although the median progression-free survival was less than 3 months and no activity was detected against brain metastases, which is a common problem in patients with ALK-rearranged NSCLC. Overall, it was concluded that luminespib had a manageable safety profile and is active in patients with both ALK rearrangement and EGFR mutations, including the gatekeeper mutant T790M, which exhibited resistance to EGFR tyrosine kinase inhibitors. Of interest was the activity of luminespib in a Phase II trial in NSCLC patients harbouring oncogenic EGFR exon 20 insertion mutants who at the time were refractory to available EGFR tyrosine kinase inhibitors, as also seen in nonclinical models (Piotrowska et al. 2018).

Ganetespib has progressed all the way to a Phase III study (known as (GALAXY-2) comparing the HSP90 inhibitor plus the taxane docetaxel versus docetaxel alone in advanced NSCLC (Pillai et al. 2017). The trial showed that there was no significant difference in median overall survival for the two arms (10.9 months for the combination versus 10.5 months with docetaxel alone. Of interest, ganetespib appeared to have lower ocular toxicity than luminepsib at the respective doses used, potentially related to the higher exposure of the retinal epithelium to the hydrophilic luminespib compared to the more hydrophobic ganetespib (see ref. (Piotrowska et al. 2018). A weakness of the trial design was that the patients were not molecularly stratified, for example to include only ALK-rearranged and EGFR-mutant. It is also possible that once or twice a week dosing may not give sufficient depletion of key oncogenic client proteins. A commentary on the study (Pillai and Ramalingam 2018) suggested that newer orally administered HSP90 inhibitors could be trialled in molecular stratified patients with NSCLC, particularly if it was possible to dose the drug so as to maintain continued suppression of the relevant HSP90 client oncoproteins. However, the authors pointed out that there are now a range of kinase inhibitors and immunotherapies in NSCLC, together with chemotherapy combinations, which may poten-

tially decrease the interest in pursuing HSP90 inhibitors in this setting at this time (Pillai and Ramalingam 2018).

In a retrospective review of 158 patients treated with various HSP90 inhibitors (tanespimycin, alvespimycin, retaspimycin, ganetespib and CNF2024) at Memorial Sloan Kettering Cancer Center, sufficient tissue to allow biomarker assessment was available for 51 patients and it is noteworthy that 13 of 16 responses strongly correlated with HER2-positive status (Jhaveri et al. 2016). The authors concluded that their findings were consistent with the preclinical data demonstrating that HER2 is the most sensitive client protein of HSP90 inhibition and suggested that this biomarker requires prospective validation in larger studies.

Overall, the experience to date with HSP90 inhibitors in the clinic is that doses can be given that provide evidence of pharmacological inhibition of HSP90. However, greater reliance on HSP72 as a PD biomarker and relatively little information on depletion of oncogenic client proteins, which requires higher exposures, means that it remains unclear whether in the trials described above the key client oncoproteins have been depleted to a sufficient extent and for adequate time to reveal the full therapeutic potential of HSP90 inhibitors. Concerning side-effects, the first-generation geldanamycin analogues exhibited liver toxicity that is potentially related to the quinone moiety – whereas ocular effects, typified by reversible night blindness, blurred vision, and flashing lights, were more of a limitation with non-geldanamycin inhibitors, to a degree that varies between the different second generation inhibitors and may relate to physical properties and retinal exposure.

In a review of HSP90 inhibitors in 2015, we focused on potential approaches to maximize the therapeutic potential of these agents (Butler et al. 2015). Possibilities included optimizing dosing and schedule through use of PD biomarkers; dissecting and exploiting the complex molecular and cellular response to HSP90 inhibition, including effects of co-chaperones; and use of combinatorial drug strategies, including the application of a one-two punch approach – employing a combination of drugs that directly inhibit the function of a key client protein (eg. kinase activity using a kinase inhibitor) together with an HSP90 inhibitor to deplete the overall protein level. Of interest in this regard is our observation that ATP-competitive protein kinase inhibitors can, as single agents, deplete the levels of the target kinase by blocking protein kinase recruitment to the HSP90-CDC37 system (Polier et al. 2013). In support of the kinase-HSP90 inhibitor one-two punch idea, it has been shown that inhibition of HSP90 by the resorcinol drug onalespib delays the emergence of resistance to BRAF kinase inhibition and overcomes resistance to dual BRAF and MEK inhibition in melanoma models (Smyth et al. 2014). In addition, relatively low level HSP90 inhibition blocks the emergence of resistance to anti-oestrogens in breast cancer models (Whitesell et al. 2014). Note also that we have shown that onalespib blocks mRNA splicing of androgen receptor variant 7 in prostate cancer cells (Ferraldeschi et al. 2016). This suggests an approach to overcome resistance mediated via the splice variant, in addition to the more conventional HSP90 inhibitor mechanism of proteasomal depletion of the wild type receptor.

Efforts continue to design novel classes of HSP90 inhibitors with distinct properties. One example is the identification of inhibitors with different HSP90 paralog selectivity profiles (Huck et al. 2019). Another is a series of inhibitors based on the novobiocin structure that bind at or close to the HSP90 C-terminal dimerization domain, potentially involving a putative second nucleotide-binding pocket, from which some analogues are reported to have reduced propensity to induce the heat shock response (Neckers et al. 2018 and see Chap. 9). In addition, we need to remain alert to the identification of cancers not considered previously that might be responsive to HSP90 inhibitors. A possible example is our recent demonstration of the promising activity of HSP90 inhibitors in nonclinical models of bile duct cancers known as cholangiocarcinomas – for which microRNA 21 (miRNA21) appears to mediate resistance by decreasing levels of the DnaJ Heat Shock Protein family (Hsp40) member B5 (DNAJB5) (Lampis et al. 2018). It was

suggested that miRNA21 could be a potential marker of sensitivity for HSP90 inhibitor sensitivity in these hard-to-treat cancers (Lampis et al. 2018).

11.3 Therapeutic Targeting of HSP90 Co-Chaperones, HSP70 and HSF1

It was mentioned above that it may be possible to exploit the complex molecular and cellular response to HSP90 inhibition to enhance therapeutic effectiveness or provide alternative therapeutic targeting. Our lab (often in collaboration with the Pearl group) and others have explored the consequences of depleting co-chaperones such as CDC37 (Smith et al. 2009, 2015), AHA1 (Holmes et al. 2008) and the E3 ubiquitin ligase Cullin-5 (Samant et al. 2014). We showed that depletion of CDC37 sensitizes cancer cells to HSP90 inhibitors and leads to client kinase depletion – but greatly reduced inhibition of binding of CDC37 to HSP90 inhibition does not. Indeed, rather surprisingly, we demonstrated that CDC37 is able to stabilise kinase clients by a mechanism that is not dependent on a substantial direct interaction between CDC37 and HSP90, but nevertheless requires HSP90 activity. These results indicate that pharmacological inhibition of CDC37-HSP90 binding is unlikely to be effective for cancer therapy (Smith et al. 2015). We also showed that knockdown of the HSP90 ATPase-promoting co-chaperone AHA1 decreases client protein activation and increases cancer cell sensitivity to the HSP90 inhibition (Smith et al. 2009). In our work with Cullin-5 (CUL5), we again obtained somewhat surprising results in that knockdown of this E3 ligase modulates multiple molecular and cellular responses to HSP90 inhibition in human cancer cells (Smith et al. 2015). CUL5 was found to be required for degradation of a number of HSP90 clients after treatment with an HSP90 inhibitor. Unexpectedly, silencing CUL5 also slows the earlier loss of HSP90 client protein activity while also delaying co-chaperone dissociation from inhibited HSP90-client complexes. In addition, depleting CUL5 decreased the sensitivity of cancer cells to three distinct HSP90 inhibitors, across four tumour types that are driven by different protein kinases. These findings show that the role of E3 ligases in the pharmacology of HSP90 inhibitors is more complex than previously considered, and not simply concerned with client protein degradation. Pharmacological modulation of E3 ligases is of course of interest. In addition CUL5 may be a potential biomarker for sensitivity to HSP90 inhibitors.

Inhibition of the heat shock response or components of it (other than HSP90 discussed above) is an alternative approach to cancer therapy in the proteostasis area. The heat shock response was of course famously discovered by Feruccio Ritossa when he observed the effect of the inadvertent increase in a lab incubator temperature on the 'puffing' of polytene chromosomes in the salivary glands of *Drosophila*, which he then linked to new RNA synthesis (Ritossa 1962, 1996). The heat shock response was subsequently shown to be mediated by heat shock transcription factor HSF1 – the major transcriptional regulator of the eukaryotic heat shock response and the primary mediator of transcriptional responses to proteotoxic stress – which binds to Heat Shock Elements in heat shock-regulated genes (Rabindran et al. 1991; Anckar and Sistonen 2011). Studies by Voellmy and colleagues showed that the heat shock response can be activated by the release of HSF1 from the inhibitory stress-sensitive complex it forms with HSP90 (Zou et al. 1998). This can be triggered by unfolded proteins binding to HSP90. The mechanisms are, however, quite complex and certainly involve trimerization, nuclear accumulation and post-translational modification of HSF1, leading to activation of induction of a fraction of heat shock genes by increasing RNA polymerase II release from promoter-proximal pause (Mahat et al. 2016).

The Whitesell lab published an important study on the effect of HSP90 inhibitors in mouse embryo fibroblasts derived from Ivor Benjamin's HSF1 knockout mice as compared with wild type controls (Bagatell et al. 2000). They showed that HSF1 knockout cells exhibited significantly

greater sensitivity to the effect of HSP90 inhibitors, which was linked to failure to activate the cytoprotective heat shock response in knockout cells in contrast to wild type cells; there was no change in response to the cytotoxic agents doxorubicin or cisplatin. Also to note is that, in the same paper, the authors demonstrated that treatment with tanespimycin induced the expression of the representative heat shock protein HSP72 both in normal mouse tissues and human tumour xenografts – an effect subsequently used as part of the pharmacodynamic biomarker signature for HSP90 inhibition in drug discovery and clinical trials (see above).

Given the challenges of inhibiting the HSF1 transcription factor directly, our lab set out to investigate whether inhibiting key components of the heat shock response (other than HSP90) could exert selective anticancer activity and also whether this approach could sensitize cancer cells to HSP90 inhibitors (Powers et al. 2008). HSP70 isoforms are known to contribute to tumorigenesis, for example through their anti-apoptotic activity and their involvement as co-chaperones for HSP90. We showed that silencing the expression of either HSP72 – a well-studied heat shock inducible gene – or heat shock cognate 70 (HSC70) in human cancer cell lines has no effect on the activity of HSP90 in chaperoning client proteins or on cell proliferation. In contrast, simultaneous combinatorial depletion of both of these isoforms induces the proteasome-dependent degradation of HSP90 client proteins, causes G1 cell-cycle arrest and triggers extensive apoptosis – the latter to a much greater extent than pharmacological HSP90 inhibition – all in the absence of HSP90 inhibitors. In contrast, combinatorial silencing of the two HSP70 isoforms in non-tumorigenic cell lines does not cause comparable growth arrest or induction of apoptosis, indicating a potential therapeutic window for this combinatorial approach. The need for HSC70 inhibition in addition to HSP72 was explained by the ability of HSC70 to replace HSP72 in the heterochaperone complex with HSP90. Sensitization to HSP90 inhibitors was also seen in cancer cells. Similar effects of dual knockdown of HSP72 and HSC70 have also been confirmed by others.

These findings provided validation for the discovery of HSP70 inhibitors. HSP70 proteins are members of the actin family of ATPases for which the ATP site is much more challenging to drug than HSP90 and which has delivered very little success in the discovery of high affinity ligands. One challenging feature is the flexibility of the ATP site, which undergoes numerous conformational changes. The Vernalis team identified novel adenosine-derived ligands through structure-based design and found selectivity towards the GRP78 isoform (Williamson et al. 2009). In our efforts to design HSP70 inhibitors we have also adopted structure-based design strategies (Cheeseman et al. 2016; Jones et al. 2016). In particular, our ICR team took the approach of exploiting protein conformational change to optimize adenosine-based inhibitors of HSP70 (Cheeseman et al. 2016). We provided evidence that such adenosine-derived HSP70 ligands have the potential to bind to the protein with a novel mechanism of action, which involves the stabilization by desolvation of an intramolecular salt-bridge that in turn induces a conformational change in HSP70, leading to high affinity ligands. We also demonstrated that through the application of this mechanism, adenosine-derived HSP70 inhibitors can be optimized in a rational manner. We concluded that improved understanding of the flexibility of HSP70 and the impact of this on the affinity of ligands should contribute to better assay design and enhanced efficiency of inhibitor optimization. We also carried out a comprehensive fragment-based exploration of an HSP70 family enzyme, resulting in the discovery of an aminoquinazoline fragment which we then elaborated to produce novel ATP site binders that exhibited physicochemical properties distinct from previously known adenosine-based HSP70 inhibitors (Jones et al. 2016). In addition, my ICR colleagues discovered an irreversible inhibitor of HSP72 that unexpectedly targets lysine-56 and enhanced this series of targeted covalent inhibitors using rational design and kinetic optimization to yield Compound 18

(Pettinger et al. 2017, 2019). Other groups have also reported HSP70 family inhibitors, in particular Gabriella Chiosis and Jason Gestwicki (Rodina et al. 2013; Gestwicki and Shao 2019). However, to date HSP70 inhibitors have not progressed to the clinic.

Recent attention has turned to the HSF1 transcription factor as a potential target for cancer therapy (Whitesell and Lindquist 2009; De Billy et al. 2009). Although the available structure of the DNA binding domain indicates that it would be extremely tough to drug, HSF1 has been strongly validated, especially by key work in the Lindquist lab, as a critical protein involved in supporting oncogenesis and the tumour state (Dai et al. 2007). Other important work by the Lindquist group showed that HSF1 drives a transcriptional programme that is distinct from heat shock and which supports the malignancy of human cancers; moreover, HSF1 expression is prognostic in several clinical cancers (Mendillo et al. 2012). In addition to cell autonomous effects, HSF1 supports the reprogramming of tumour stroma, contributing to malignancy (Scherz-Shouval et al. 2014).

Recognising the druggability challenge posed by HSF1, we carried out a mechanism-based phenotypic screen to identify small-molecule inhibitors of the HSF1-mediated heat shock response; through this we discovered the bisamide amide class of inhibitors that exhibit a potent and selective effect on the HSF1 pathway in intact cancer cells (Cheeseman et al. 2017). We optimized this series, in particular to enhance intrinsic solubility, resulting in the advanced lead compound CCT251236 which showed therapeutic activity in models of human ovarian cancer and multiple myeloma (Cheeseman et al. 2017; Fok et al. 2018). Using chemical proteomics and validated by biophysical methods of surface plasmon resonance and x-ray crystallography, we identified the putative co-transcriptional regulator pirin as a molecular target that may be responsible for the anti-migration properties of CCT251236 (Cheeseman et al. 2017). Moreover, we designed and synthesized a pirin degrader (CCT367766) which showed potent and specific depletion of pirin in cancer cells; this can be used to demonstrate in-cell target engagement with pirin (Chessum et al. 2018). Meanwhile we continue to search for additional molecular targets of CCT251236 while progressing a clinical candidate towards first-in-human studies.

11.4 The Importance of Chemical Probes

CCT251236 has been rated positively as a 'chemical probe' for pirin at the Chemical Probes Portal (The Chemical Probes Portal n.d.). High-quality chemical probes are important reagents, used alongside genetic and biochemical tools, for probing biology and disease mechanisms and validating drug targets (Workman and Collins 2010; Frye 2010). A number of small-molecules discussed herein and also other compounds are important chemical probes or 'tools' in the chaperone/proteostasis area (Gestwicki and Shao 2019; Shrestha et al. 2016). To be useful, and not misleading, high-quality chemical probes must exhibit essential features or 'fitness factors' – particularly high potency and selectivity towards the desired target, together with cellular permeability and evidence of modulation of the target in cells (Workman and Collins 2010; Frye 2010). Unfortunately, the literature is full of examples of the use of chemical agents that lack the characteristics of high quality chemical probes and may even act broadly as chemically reactive compounds or behave as detergents or aggregators. Compounds such as apoptozole and 2-phenylethynesulfonamide (also known as PES and pifithrin-μ) have been used as chemical probes for HSP70 proteins but have subsequently been shown to be very non-specific (Evans et al. 2015; Schlecht et al. 2013).

My colleagues and I at ICR have provided chemical probes for use by the community, and have also contributed to guidelines for best practice in the selection and use of chemical probes for biological and biomedical research (Workman and Collins 2010; Arrowsmith et al. 2015; Blagg and Workman 2017). We are now hosting the Chemical Probes Portal – a public resource pro-

viding expert peer-viewed recommendations for chemical probes (Arrowsmith et al. 2015). We have also developed a public resource called Probe Miner (Antolin et al. 2018) that is complementary to the Portal in providing objective, quantitative, data-driven assessment of chemical probes, building on our canSAR knowledgebase (Coker et al. 2019). In addition, we recently provided an overview of the public resources available to help in the selection of chemical probes – rather than relying on the literature, general search engines and vendor catalogues that are biased in various ways and lack annotation – and we also included advice on how to navigate the various resources to make an informed choice of chemical probes (Antolin et al. 2019).

11.5 Concluding Remarks and Future Outlook

I hope this personal view has provided an insight into how findings in basic research on proteostasis networks can provide chemical probes and drug candidates for use in oncology research and cancer treatment. I also very much hope that the HSF1 pathway inhibitor will fulfil its potential in showing clinical activity in ovarian cancer – the hard-to-treat disease that cruelly took Sue Lindquist from us way too early.

Acknowledgements I acknowledge grant support from Cancer Research UK (CRUK) [C309/A11566] and Wellcome [212969/Z/18/Z] and I am a CRUK Life Fellow. I would like to thank many lab members, colleagues and collaborators for their invaluable contributions and discussions and to Dr. Matt Cheeseman for preparing Figure 1. Like many others, I am also indebted to Sue Lindquist for her outstanding leadership, inspiration and research contributions. This article is a brief personal perspective and I apologize that many excellent publications could not be cited.

Conflict of Interest PW is Chief Executive, President and an employee of the Institute of Cancer Research (ICR) which has a commercial interest in a range of drug targets, including HSP90 and HSF1. The ICR operates a Rewards to Inventors scheme whereby employees of the ICR may receive financial benefit following commercial licensing of a project. PW received research funding relevant to this article from Vernalis, Astex, AstraZeneca, BACIT and Sixth Element Capital/CRT Pioneer Fund. PW contributed to intellectual property on HSP90 inhibitors that was licenced to Vernalis and Novartis and on HSF1 that was licenced to Sixth Element Capital/CRT Pioneer Fund. PW has been a consultant to Novartis, a consultant/scientific advisory board member for NextechInvest, Storm Therapeutics, Astex Pharmaceuticals and CV6, and holds stock in Chroma Therapeutics, NextInvest and Storm Therapeutics. He is also a Non-Executive Director of Storm Therapeutics and the Royal Marsden NHS Trust and a Board Director of the non-profit Chemical Probes Portal.

References

Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu Rev Biochem 80:1089–1115

Antolin AA, Tym JE, Komianou A, Collins I, Workman P, Al-Lazikani B (2018) Objective, quantitative, data-driven assessment of chemical probes. Cell Chem Biol 25(2):194–205.e5

Antolin A, Workman P, Al-Lazikani B (2019) Public resources for chemical probes: the journey so far and the road ahead. Future Med Chem [Online ahead of print]

Arrowsmith CH, Audia JE, Austin C, Baell J, Bennett J, Blagg J, Bountra C, Brennan PE, Brown PJ, Bunnage ME, Buser-Doepner C, Campbell RM, Carter AJ, Cohen P, Copeland RA, Cravatt B, Dahlin JL, Dhanak D, Edwards AM, Frederiksen M, Frye SV, Gray N, Grimshaw CE, Hepworth D, Howe T, Huber KV, Jin J, Knapp S, Kotz JD, Kruger RG, Lowe D, Mader MM, Marsden B, Mueller-Fahrnow A, Müller S, O'Hagan RC, Overington JP, Owen DR, Rosenberg SH, Roth B, Ross R, Schapira M, Schreiber SL, Shoichet B, Sundström M, Superti-Furga G, Taunton J, Toledo-Sherman L, Walpole C, Walters MA, Willson TM, Workman P, Young RN, Zuercher WJ (2015) The promise and peril of chemical probes. Nat Chem Biol 11:536–541

Arteaga CL (2011) Why is this effectiveHSP90 inhibitor not being developed in HER2+ breast cancer? Clin Cancer Res 17:4919–4921

Bagatell R, Paine-Murrieta GD, Taylor CW, Pulcini EJ, Akinaga S (2000) Benjamin IJ Whitesell L. Induction of a Heat Shock Factor 1-dependent stress response alters the cytotoxic activity of Hsp90-binding agents. Clin Cancer Res 6:3312–3318

Banerji U, Workman P (2016) Critical parameters in targeted drug development: the pharmacological audit trail. Semin Oncol 43:436–445

Banerji U, Walton M, Raynaud F, Grimshaw R, Kelland L, Valenti M, Judson I, Workman P (2005a) Pharmacokinetic-pharmacodynamic relationships for the heat shock protein 90 molecular chaperone inhibitor 17-allylamino, 17-demethoxygeldanamycin in

human ovarian cancer xenograft models. Clin Cancer Res 11:7023–7032

Banerji U, O'Donnell A, Scurr M, Pacey S, Stapleton S, Asad Y, Simmons L, Maloney A, Raynaud F, Campbell M, Walton M, Lakhani S, Kaye S, Workman P, Judson I (2005b) Phase I pharmacokinetic and pharmacodynamic study of 17-allylamino,17-demethoxygeldanamycin in patients with advanced malignancies. J Clin Oncol 23:4152–4161

Banerji U, Affolter A, Judson I, Marais R, Workman P (2008) BRAF and NRAS mutations in melanoma: potential relationships to clinical response to HSP90 inhibitors. Mol Cancer Ther 7:737–739

Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, Reddy A, Liu M, Murray L, Berger MF, Monahan JE, Morais P, Meltzer J, Korejwa A, Jané-Valbuena J, Mapa FA, Thibault J, Bric-Furlong E, Raman P, Shipway A, Engels IH, Cheng J, Yu GK, Yu J, Aspesi P Jr, de Silva M, Jagtap K, Jones MD, Wang L, Hatton C, Palescandolo E, Gupta S, Mahan S, Sougnez C, Onofrio RC, Liefeld T, MacConaill L, Winckler W, Reich M, Li N, Mesirov JP, Gabriel SB, Getz G, Ardlie K, Chan V, Myer VE, Weber BL, Porter J, Warmuth M, Finan P, Harris JL, Meyerson M, Golub TR, Morrissey MP, Sellers WR, Schlegel R, Garraway LA (2012) The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483:603–607

Basso AD, Solit DB, Munster PN, Rosen N (2002) Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. Oncogene 21:1159–1166

Blagg J, Workman P (2017) Choose and use your chemical probe wisely to explore cancer biology. Cancer Cell 32:9–25

Brough PA, Aherne W, Barril X, Borgognoni J, Boxall B, Cansfield JE, Cheung KM, Collins I, Davies NGM, Drysdale MJ, Dymock B, Eccles SA, Finch H, Fink A, Hayes A, Howes R, Hubbard RE, James K, Jordan AM, Lockie A, Martins V, Massey A, Matthews T, McDonald E, Northfield CJ, Pearl LH, Prodromou C, Ray S, Raynaud FI, Roughley SD, Sharp SY, Surgenor A, Walmsley DL, Webb P, Wood M, Workman P, Wright L (2008) 4,5-Diaryl isoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. J Med Chem 51:196–218

Brough PA, Barril X, Borgognoni J, Chene P, Davies NGM, Davis B, Drysdale MJ, Dymock B, Eccles SA, Garcia-Echeverria C, Fromont C, Hayes A, Hubbard RE, Jordan AM, Jensen MR, Massey A, Merrett A, Padfield A, Parsons R, Radimerski T, Raynaud FI, Robertson A, Roughley SD, Schoepfer J, Simmonite H, Sharp SY, Surgenor A, Valenti M, Walls S, Webb P, Wood M, Workman P, Wright L (2009) Combining hit identification strategies: fragment-based and in silico approaches to orally active 2-aminothieno[2,3-d]pyrimidine inhibitors of the Hsp90 molecular chaperone. J Med Chem 52:4794–4809

Brunton VG, Workman P (1993) Cell-signaling targets for antitumour drug development. Cancer Chemother Pharmacol 32:1–19

Brunton VG, Steele G, Lewis AD, Workman P (1998) Geldanamycin-induced cytotoxicity in human colon-cancer cell lines: evidence against the involvement of c-Src or DT-diaphorase. Cancer Chemother Pharmacol 41:417–422

Butler LM, Ferraldeschi R, Armstrong HK, Centenera MM, Workman P (2015) Maximizing the therapeutic potential of HSP90 inhibitors. Mol Cancer Res 13:1445–1451

Cartwright CA, Meisler AI, Eckhart W (1990) Activation of the pp60c-src protein kinase is an early event in colon carcinogenesis. Proc Natl Acad Sci U S A 87:558–562

Cheeseman MD, Westwood IM, Barbeau O, Rowlands M, Dobson S, Jones AM, Jeganathan F, Burke R, Kadi N, Workman P, Collins I, van Montfort RL, Jones K (2016) Exploiting protein conformational change to optimize adenosine-derived inhibitors of HSP70. J Med Chem 59:4625–4636

Cheeseman MD, Chessum NE, Rye CS, Pasqua AE, Tucker MJ, Wilding B, Evans LE, Lepri S, Richards M, Sharp SY, Ali S, Rowlands M, O'Fee L, Miah A, Hayes A, Henley AT, Powers M, Te Poele R, De Billy E, Pellegrino L, Raynaud F, Burke R, van Montfort RL, Eccles SA, Workman P, Jones K (2017) Discovery of a chemical probe bisamide (CCT251236): an orally bioavailable efficacious pirin ligand from a Heat Shock Transcription Factor 1 (HSF1) phenotypic screen. J Med Chem 60:180–120

Chessum NEA, Sharp SY, Caldwell JJ, Pasqua AE, Wilding B, Colombano G, Collins I, Ozer B, Richards M, Rowlands M, Stubbs M, Burke R, McAndrew PC, Clarke PA, Workman P, Cheeseman MD, Jones K (2018) Demonstrating in-cell target engagement using a pirin protein degradation probe (CCT367766). J Med Chem 61(3):918–933

Cheung KM, Matthews TP, James K, Rowlands MG, Boxall KJ, Sharp SY, Maloney A, Roe SM, Prodromou C, Pearl LH, Aherne GW, McDonald E, Workman P (2005) The identification, synthesis, protein crystal structure and in vitro biochemical evaluation of a new 3,4-diarylpyrazole class of Hsp90 inhibitors. Bioorg Med Chem Lett 15:3338–3343

Chiosis G, Timaul MN, Lucas B, Munster PN, Zheng FF, Sepp-Lorenzino L, Rosen N (2001) A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. Chem Biol 8:289–299

Clarke PA, Hostein I, Banerji U, Di Stefano F, Maloney A, Walton M, Judson I, Workman P (2000) Gene expression profiling of human colon adenocarcinoma cells following inhibition of signal transduction by 17-allylamino-17-demethoxyl-geldanamycin, an inhibitor of the hsp90 molecular chaperone. Oncogene 19:4125–4133

Coker EA, Mitsopoulos C, Tym JE, Komianou A, Kannas C, Di Micco P, Villasclaras Fernandez E, Ozer B, Antolin AA, Workman P, Al-Lazikani B (2019) canSAR: update to the cancer translational research and drug discovery knowledgebase. Nucleic Acids Res 47(D1):D917–D922

da Rocha Dias S, Light Y, Friedlos F, Springer C, Workman P, Marais R (2005) Oncogenic B-RAF is an Hsp90 client protein that is targeted by the anticancer drug 17-AAG. Cancer Res 65:10686–10691

Dai C, Whitesell L, Rogers AB, Lindquist S (2007) Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. Cell 130:1005–1001

De Billy E, Powers MV, Smith JR, Workman P (2009) Drugging the heat shock factor 1 pathway: exploitation of the critical cancer cell dependence on the guardian of the proteome. Cell Cycle 8:3806–3808

Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung KM, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P (2008) NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. Cancer Res 68:2850–2860

Evans LE, Cheeseman MD, Yahya N, Jones K (2015) Investigating apoptozole as a chemical probe for HSP70 inhibition. PLoS One 10:e0140006

Felip E, Barlesi F, Besse B et al (2018) Phase 2 study of the HSP-90 inhibitor AUY922 in previously treated and molecularly defined patients with advanced non-small cell lung cancer. J Thorac Oncol 13:579–587

Ferraldeschi R, Welti J, Powers MV, Yuan W, Smyth T, Seed G, Riisnaes R, Hedayat S, Wang H, Crespo M, Nava Rodrigues D, Figueiredo I, Miranda S, Carreira S, Lyons JF, Sharp S, Plymate SR, Attard G, Wallis N, Workman P, de Bono JS (2016) Second-generation Hsp90 inhibitor onalespib blocks mRNA splicing of androgen receptor variant 7 in prostate cancer cells. Cancer Res 76:2731–2742

Fitzsimmons SA, Workman P, Grever M, Paull K, Camelier R, Lewis AD (1996) Reductase expression across the National Cancer Institute tumor cell line panel: correlation with sensitivity to mitomycin C and EO9. J Natl Cancer Inst 88:259–269

Fok JHL, Hedayat S, Zhang L, Aronson LI, Mirabella F, Pawlyn C, Bright MD, Wardell CP, Keats JJ, De Billy E, Rye CS, Chessum NEA, Jones K, Morgan GJ, Eccles SA, Workman P, Davies FE (2018) HSF1 is essential for myeloma cell survival and a promising therapeutic target. Clin Cancer Res 24:2395–2407

Frye SV (2010) The art of the chemical probe. Nat Chem Biol 6:159–161

Garcia R, Parikh NU, Saya H, Gallick GE (1991) Effect of herbimycin A on growth and pp60c-src activity in human colon tumor cell lines. Oncogene 6:1983–1989

Garcia-Carbonero R, Carnero A, Paz-Ares L (2013) Inhibition of HSP90 molecular chaperones: moving into the clinic. Lancet Oncol 14:e358–e369

Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, Greninger P, Thompson IR, Luo X, Soares J, Liu Q, Iorio F, Surdez D, Chen L, Milano RJ, Bignell GR, Tam AT, Davies H, Stevenson JA, Barthorpe S, Lutz SR, Kogera F, Lawrence K, McLaren-Douglas A, Mitropoulos X, Mironenko T, Thi H, Richardson L, Zhou W, Jewitt F, Zhang T, O'Brien P, Boisvert JL, Price S, Hur W, Yang W, Deng X, Butler A, Choi HG, Chang JW, Baselga J, Stamenkovic I, Engelman JA, Sharma SV, Delattre O, Saez-Rodriguez J, Gray NS, Settleman J, Futreal PA, Haber DA, Stratton MR, Ramaswamy S, McDermott U, Benes CH (2012) Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483:570–575

Gestwicki JE, Shao H (2019) Inhibitors and chemical probes for molecular chaperone networks. J Biol Chem 294:2151–2216

Grbovic OM, Basso AD, Sawai A, Ye Q, Friedlander P, Solit D, Rosen N (2006) V600E B-Raf requires the Hsp90 chaperone for stability and is degraded in response to Hsp90 inhibitors. Proc Natl Acad Sci U S A 103:57–62

Guo W, Reigan P, Siegel D, Zirrolli J, Gustafson D, Ross D (2005) Formation of 17-allylamino-demethoxygeldanamycin (17-AAG) hydroquinone by NAD(P)H:quinoneoxidoreductase1: role of 17-AAG hydroquinone in heat shock protein 90 inhibition. Cancer Res 65:10006–10015

Holmes JL, Sharp SY, Hobbs S, Workman P (2008) Silencing of HSP90 cochaperone AHA1 expression decreases client protein activation and increases cellular sensitivity to the HSP90 inhibitor 17-allyl amino-17-demethoxygeldanamycin. Cancer Res 68:1188–1197

Hostein I, Robertson D, Di Stefano F, Workman P, Clarke PA (2001) Inhibition of signal transduction by the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin results in cytostasis and apoptosis. Cancer Res 61:4003–4009

HSP90 Interactors (n.d.) www.picard.ch/downloads/Hsp90interactors.pdf

Huck JD, Que NLS, Sharma S, Taldone T, Chiosis G, Gewirth DT (2019) Structures of Hsp90α and Hsp90β bound to a purine-scaffold inhibitor reveal an exploitable residue for drug selectivity. Proteins 87:869–877

Jhaveri K, Chandarlapaty S, Iyengar N, Morris P, Corben A, Patil S, Akram Towers R, Sakr R, King TA, Norton L, Rosen N, Hudis C, Modi S (2016) Biomarkers that predict sensitivity to Heat Shock Protein 90 inhibitors. Clin Breast Cancer 16:276–278

Jones AM, Westwood IM, Osborne JD, Matthews TP, Cheeseman MD, Rowlands MG, Jeganathan F, Burke R, Lee D, Kadi N, Liu M, Richards M, McAndrew C,

Yahya N, Dobson SE, Jones K, Workman P, Collins I, van Montfort RL (2016) A fragment-based approach applied to a highly flexible target: insights and challenges towards the inhibition of HSP70 isoforms. Sci Rep 6:34701

Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P (1999) DT-diaphorase expression and tumor cell sensitivity to 17- allylamino,17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. J Natl Cancer Inst 91:1940–1949

Kong A, Rea D, Ahmed S, Beck JT, López López R, Biganzoli L, Armstrong AC, Aglietta M, Alba E, Campone M, Hsu Schmitz SF, Lefebvre C, Akimov M, Lee SC (2016) Phase 1B/2 study of the HSP90 inhibitor AUY922 plus trastuzumab in metastatic HER2-positive breast cancer patients who have progressed on trastuzumab-based regimen. Oncotarget 7:37680–33769

Lampis A, Carotenuto P, Vlachogiannis G, Cascione L, Hedayat S, Burke R, Clarke P, Bosma E, Simbolo M, Scarpa A, Yu S, Cole R, Smyth E, Mateos JF, Begum R, Hezelova B, Eltahir Z, Wotherspoon A, Fotiadis N, Bali MA, Nepal C, Khan K, Stubbs M, Hahne JC, Gasparini P, Guzzardo V, Croce CM, Eccles S, Fassan M, Cunningham D, Andersen JB, Workman P, Valeri N, Braconi C (2018) MIR21 drives resistance to Heat Shock Protein 90 inhibition in cholangiocarcinoma. Gastroenterology 154:1066–1079

Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT (2016) Mammalian Heat Shock Response and mechanisms underlying its genome-wide transcriptional regulation. Mol Cell 62:63–78

Maloney A, Clarke PA, Naaby-Hansen S, Stein R, Koopman JO, Akpan A, Yang A, Zvelebil M, Cramer R, Stimson L, Aherne W, Banerji U, Judson I, Sharp S, Powers M, de Billy E, Salmons J, Walton M, Burlingame A, Waterfield M, Workman P (2007) Gene and protein expression profiling of human ovarian cancer cells treated with the heat shock protein 90 inhibitor 17-allylamino-17-demethoxygeldanamycin. Cancer Res 67:3239–3253

Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, Fraenkel E, Ince TA, Whitesell L, Lindquist S (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. Cell 150:549–562

Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, D'Andrea G, Dickler M, Moynahan ME, Sugarman S, Ma W, Patil S, Norton L, Hannah AL, Hudis C (2011) HSP90 inhibition is effective in breast cancer: a phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. Clin Cancer Res 17:5132–5139

Münster PN, Srethapakdi M, Moasser MM, Rosen N (2001) Inhibition of heat shock protein 90 function by ansamycins causes the morphological and functional differentiation of breast cancer cells. Cancer Res 61:2945–2952

Neckers L, Workman P (2012) Hsp90 molecular chaperone inhibitors: are we there yet? Clin Cancer Res 18:64–76

Neckers L, Blagg B, Haystead T, Trepel JB, Whitesell L, Picard D (2018) Methods to validate Hsp90 inhibitor specificity, to identify off-target effects, and to rethink approaches for further clinical development. Cell Stress Chaperones 23:467–482

Pettinger J, Le Bihan YV, Widya M, van Montfort RL, Jones K, Cheeseman MD (2017) An irreversible inhibitor of HSP72 that unexpectedly targets lysine-56. Angew Chem Int Ed Engl 56:3536–3540

Pettinger J, Carter M, Jones K, Cheeseman MD (2019) Kinetic optimization of lysine-targeting covalent inhibitors of HSP72. J Med Chem 62:11383–11398.

Pillai RN, Ramalingam S (2018) Throwing more cold water on Heat Shock Protein 90 inhibitors in NSCLC. J Thorac Oncol 13:473–474

Pillai R, Fennell D, Kovcin V, Ciuleanu T, Ramlau R, Kowalski D, Schenker M, Perin B, Yalcin I, Teofilovici F, Vukovic V, Ramalingam S (2017) Phase 3 study of ganetespib, a heat shock protein 90 inhibitor, with docetaxel versus docetaxel in advanced non-small cell lung cancer (GALAXY-2). J Thorac Oncol 12:S7–S8

Piotrowska Z, Costa DB, Oxnard GR, Huberman M, Gainor JF, Lennes IT, Muzikansky A, Shaw AT, Azzoli CG, Heist RS, Sequist LV (2018) Activity of the Hsp90 inhibitor luminespib among non-small-cell lung cancers harboring EGFR exon 20 insertions. Ann Oncol 29:2092–2020

Polier S, Samant RS, Clarke PA, Workman P, Prodromou C, Pearl LH (2013) ATP-competitive inhibitors block protein kinase recruitment to the Hsp90-Cdc37 system. Nat Chem Biol 9:307–312

Powers MV, Clarke PA, Workman P (2008) Dual targeting of HSC70 and HSP72 inhibits HSP90 function and induces tumor-specific apoptosis. Cancer Cell 14:250–262

Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 90:65–75

Rabindran SK, Giorgi G, Clos J, Wu C (1991) Molecular cloning and expression of a human heat shock factor, HSF1. Proc Natl Acad Sci U S A 88:6906–6910

Ritossa FA (1962) New puffing pattern induced by temperature Shock and DNP in Drosophila. Experientia 18:71–573

Ritossa F (1996) Discovery of the heat shock response. Cell Stress Chaperones 1:97–98

Rodina A, Patel PD, Kang Y, Patel Y, Baaklini I, Wong MJ, Taldone T, Yan P, Yang C, Maharaj R, Gozman A, Patel MR, Patel HJ, Chirico W, Erdjument-Bromage H, Talele TT, Young JC, Chiosis G (2013) Identification of an allosteric pocket on human hsp70 reveals a mode of inhibition of this therapeutically important protein. Chem Biol 20:1469–1480

Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1999) Structural basis for inhibition

of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem 42:260–266

Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D (2000) NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact 129:77–97

Samant RS, Clarke PA, Workman P (2014) E3 ubiquitin ligase Cullin-5 modulates multiple molecular and cellular responses to heat shock protein 90 inhibition in human cancer cells. Proc Natl Acad Sci U S A 111:6834–6839

Sartorelli AC (1988) Therapeutic attack of hypoxic cells of solid tumors: presidential address. Cancer Res 48:775–778

Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, Beck AH, Dias-Santagata D, Koeva M, Stemmer SM, Whitesell L, Lindquist S (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. Cell 158:564–578

Schlecht R, Scholz SR, Dahmen H, Wegener A, Sirrenberg C, Musil D, Bomke J, Eggenweiler HM, Mayer MP, Bukau B (2013) Functional analysis of Hsp70 inhibitors. PLoS One 8:e78443

Schroder CP, Pedersen JV, Chua S, Swanton C, Akimov M, Ide S et al (2011) Use of biomarkers and imaging to evaluate the treatment effect of AUY922, an HSP90 inhibitor, in patients with HER2+ or ER+ metastatic breast cancer. J Clin Oncol 29(suppl):abstr e11024

Sessa C, Shapiro GI, Bhalla KN, Britten C, Jacks KS, Mita M, Papadimitrakopoulou V, Pluard T, Samuel TA, Akimov M, Quadt C, Fernandez-Ibarra C, Lu H, Bailey S, Chica S, Banerji U (2013) First-in-human phase I dose-escalation study of the HSP90 inhibitor AUY922 in patients with advanced solid tumors. Clin Cancer Res 19:3671–3680

Sharp SY, Boxall K, Rowlands M, Prodromou C, Roe SM, Maloney A, Powers M, Clarke PA, Box G, Sanderson S, Patterson L, Matthews TP, Cheung KM, Ball K, Hayes A, Raynaud F, Marais R, Pearl L, Eccles S, Aherne W, McDonald E, Workman P (2007a) In vitro biological characterization of a novel, synthetic diaryl pyrazole resorcinol class of heat shock protein 90 inhibitors. Cancer Res 67:2206–2216

Sharp SY, Prodromou C, Boxall K, Powers MV, Holmes JL, Box G, Matthews TP, Cheung KM, Kalusa A, James K, Hayes A, Hardcastle A, Dymock B, Brough PA, Barril X, Cansfield JE, Wright L, Surgenor A, Foloppe N, Hubbard RE, Aherne W, Pearl L, Jones K, McDonald E, Raynaud F, Eccles S, Drysdale M, Workman P (2007b) Inhibition of the heat shock protein 90 molecular chaperone in vitro and in vivo by novel, synthetic, potent resorcinylic pyrazole/isoxazole amide analogues. Mol Cancer Ther 6:1198–1211

Shrestha L, Patel HJ, Chiosis G (2016) Chemical tools to investigate mechanisms associated with HSP90 and HSP70 in disease. Cell Chem Biol 23:158–172

Smith JR, Clarke PA, de Billy E, Workman P (2009) Silencing the cochaperone CDC37 destabilizes kinase clients and sensitizes cancer cells to HSP90 inhibitors. Oncogene 28:157–169

Smith JR, deBilly E, Hobbs S, Powers M, Prodromou C, Pearl L, Clarke PA, Workman P (2015) Restricting direct interaction of CDC37 with HSP90 does not compromise chaperoning of client proteins. Oncogene 34:15–26

Smyth T, Paraiso KH, Hearn K, Rodriguez-Lopez AM, Munck JM, Haarberg HE, Sondak VK, Thompson NT, Azab M, Lyons JF, Smalley KSM, Wallis NG (2014) Inhibition of HSP90 by AT13387 delays the emergence of resistance to BRAF inhibitors and overcomes resistance to dual BRAF and MEK inhibition in melanoma models. Mol Cancer Ther 13:2793–2804

Solit DB, Zheng FF, Drobnjak M, Münster PN, Higgins B, Verbel D, Heller G, Tong W, Cordon-Cardo C, Agus DB, Scher HI, Rosen N (2002) 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. Clin Cancer Res 8:986–993

Speranza G, Anderson L, Chen AP, Do K, Eugeni M, Weil M, Rubinstein L, Majerova E, Collins J, Horneffer Y, Juwara L, Zlott J, Bishop R, Conley BA, Streicher H, Tomaszewski J, Doroshow JH, Kummar S (2018) First-in-human study of the epichaperome inhibitor PU-H71: clinical results and metabolic profile. Investig New Drugs 36:230–223

Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, Lindquist S (2012) Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 150:987–1001

The Chemical Probes Portal (n.d.) https://www.chemicalprobes.org/cct251236

Travers J, Sharp S, Workman P (2012) HSP90 inhibition: two-pronged exploitation of cancer dependencies. Drug Discov Today 17:242–252

Walton MI, Bibby MA, Double JA, Plumb JA, Workman P (1992) DT-diaphorase activity correlates with sensitivity to the indoloquinone EO9 in mouse and human colon carcinomas. Eur J Cancer 28A:1597–1600

Whitesell L, Lindquist S (2009) Inhibiting the transcription factor HSF1 as an anticancer strategy. Expert Opin Ther Targets 13:469–478

Whitesell L, Mimnaugh EG, DeCosta B, Myers CE, Neckers LM (1994) Inhibition of the heat-shock protein hsp90-pp60 (v-Src) heteroprotein complex-formation by benzoquinone ansamycins – essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A 91:8324–8328

Whitesell L, Santagata S, Mendillo ML, Lin NU, Proia DA, Lindquist S (2014) HSP90 empowers evolution of resistance to hormonal therapy in human breast cancer models. Proc Natl Acad Sci U S A 111:18297–18302

Williamson DS, Borgognoni J, Clay A, Daniels Z, Dokurno P, Drysdale MJ, Foloppe N, Francis GL, Graham CJ, Howes R, Macias AT, Murray JB, Parsons R, Shaw T, Surgenor AE, Terry L, Wang Y, Wood M, Massey AJ (2009) Novel adenosine-derived inhibi-

tors of 70 KDa heat shock protein, discovered through structure-based design. J Med Chem 52:1510–1513

Workman P (2003) Auditing the pharmacological accounts for Hsp90 molecular chaperone inhibitors: unfolding the relationship between pharmacokinetics and pharmacodynamics. Mol Cancer Ther 2:131–138

Workman P, Collins I (2010) Probing the probes: fitness factors for small molecule tools. Chem Biol 17:561–567

Workman P, Stratford IR (1993) The experimental development of bioreductive drugs and their role in cancer therapy. Cancer Metastasis Rev 12:73–82

Workman P, Burrows F, Neckers L, Rosen N (2007) Drugging the cancer chaperone HSP90: combinatorial therapeutic exploitation of oncogene addiction and tumor stress. Ann N Y Acad Sci 1113:202–216

Workman P, Clarke PA, Al-Lazikani B (2012) Patient-predictive panel power. Cancer Cell 21:455–458

Ying W, Du Z, Sun L, Foley KP, Proia DA, Blackman RK, Zhou D, Inoue T, Tatsuta N, Sang J, Ye S, Acquaviva J, Ogawa LS, Wada Y, Barsoum J, Koya K (2012) Ganetespib, a unique triazolone-containing Hsp90 inhibitor, exhibits potent antitumor activity and a superior safety profile for cancer therapy. Mol Cancer Ther 11:475–484

Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 94:471–480

Correction to: Reflections and Outlook on Targeting HSP90, HSP70 and HSF1 in Cancer: A Personal Perspective

Paul Workman

Correction to:
Chapter 11 in: M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_11

The updated version of this chapter can be found at
https://doi.org/10.1007/978-3-030-40204-4_11

M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4_12

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M. L. Mendillo et al. (eds.), *HSF1 and Molecular Chaperones in Biology and Cancer*, Advances in Experimental Medicine and Biology 1243, https://doi.org/10.1007/978-3-030-40204-4