Published in final edited form as:

Adv Exp Med Biol. 2020 ; 1243: 87–99. doi:10.1007/978-3-030-40204-4_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.

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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.

Keywords

Protein network connectivity; Chaperome networks; Protein network vulnerability; Epichaperome; Anti-cancer therapy; HSP90 inhibitors; PU-H71

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 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 (HSP90 α and HSP90 β) 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 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 HSP90 α/β , CDC37 and two immunophilins (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 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 HSP90 α and HSP90 β 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 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. Hirsch/Monique Weill-Caulier Trust and Unravel Pediatric Cancer Foundation.

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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

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

Fig. 6.3.
Paradigm for a chaperome network-driven approach to cancer therapy