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Review

Approaches for defining the Hsp90-dependent proteome ☆

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ABSTRACT

Hsp90 is the target of ongoing drug discovery studies seeking new compounds to treat cancer, neurodegenerative diseases, and protein folding disorders. To better understand Hsp90's roles in cellular pathologies and in normal cells, numerous studies have utilized proteomics assays and related high-throughput tools to characterize its physical and functional protein partnerships. This review surveys these studies, and summarizes the strengths and limitations of the individual attacks. We also include downloadable spreadsheets compiling all of the Hsp90-interacting proteins identified in more than 23 studies. These tools include cross-references among gene aliases, human homologues of yeast Hsp90-interacting proteins, hyperlinks to database entries, summaries of canonical pathways that are enriched in the Hsp90 interactome, and additional bioinformatic annotations. In addition to summarizing Hsp90 proteomics studies performed to date and the insights they have provided, we identify gaps in our current understanding of Hsp90-mediated proteostasis. This article is part of a Special Issue entitled: Heat Shock Protein 90 (HSP90).

1. Introduction

Hsp90 is a molecular chaperone that is required for the viability of eukaryotic cells [1,2]. It is frequently described as the core component of a multimeric chaperone machine that functions in the folding, maturation, stabilization and activation of other proteins. Hsp90 function requires the binding and hydrolysis of ATP, which drives it through a reaction cycle that appears to involve approximately four distinct conformations [3–5]. Hsp90 works in concert with a cohort of co-chaperones that modulate its binding and hydrolysis of ATP and its interaction with protein substrates (a.k.a. "clients") [6–9]. Since these topics are covered in depth in other submissions to this special edition, we will forgo a detailed discussion of Hsp90's reaction cycle and the regulatory roles of its co-chaperones.

Most of the 200 plus proteins that have been found to interact with Hsp90 were discovered to do so serendipitously, with Hsp90 and the client co-purifying as a complex, or co-precipitating in antibody pull-down assays. The discovery that geldanamycin [10] and other compounds are highly specific Hsp90 inhibitors further advanced the field, and small molecule Hsp90 inhibitors have become invaluable tools for dissecting the functional significance of Hsp90's interactions with other proteins [7,11–14].

Because a comprehensive list of Hsp90's client proteins and regulatory subunits is available at the web site maintained by Dr. Didier Picard (http://www.picard.ch/downloads/downloads.htm), we will not attempt to duplicate information available therein. Rather, this review will concentrate on just those studies that have utilized high throughput (HTP[1]) approaches to define the Hsp90 interacting proteome, in the absence of a priori targets. Much of this discussion will center on proteomics techniques, reflecting their potential and prominence in assessing proteomes and protein interactions. Our discussion will also include findings from powerful yeast studies directed toward understanding the breadth and depth of Hsp90's roles in supporting the cellular proteome. Although mass spectrometry has been used to identify posttranslational modifications on specific individual proteins, this review will only encompass proteomics studies, defined here as studies directed toward whole sets of proteins. Below, we will introduce the HTP techniques that have been used to study Hsp90, touching upon the general strengths and weaknesses of each approach. We will then briefly survey results from studies that have used these techniques. Finally, we will attempt a synthesis of these findings and a description of how these studies have changed our perceptions of this fascinating protein. The reader's attention is also directed toward the Hsp90 interaction mapping initiatives from the laboratories of Houry [15–17], Frydman [18], and Picard [19].

2. Overview of techniques

2.1. Hsp90 interactions

Most HTP studies of Hsp90 function are directed at identifying Hsp90-interacting genes or gene products. However, this very term "Hsp90-interacting" merits consideration. An Hsp90 interaction can be a functional interaction, wherein manipulation of Hsp90 function

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impacts the Hsp90-interacting gene product or gene function. On one end of the spectrum, this functional impact might be direct, wherein compromised Hsp90 function manifests as a change in the expression of an Hsp90-interacting protein (e.g., compromised expression of that protein on a Western blot) or in some direct measure of its function (e.g., cellular phosphotyrosine content). On the other end of the spectrum, a functional impact might be further removed, manifesting as alterations in cellular growth, cell survival, or the phenotype of the organism or cell. In this manifestation, "Hsp90 interaction" would describe a genetic interaction between Hsp90 and the Hsp90-interacting gene/gene product. Typically, a detailed biochemical dissection is required to determine the extent to which functional interactions between Hsp90 and other genes or gene products are direct, versus indirect interactions that are mediated by intermediary Hsp90-dependent gene products.

Alternatively, "Hsp90 interaction" might describe the direct physical association of Hsp90 with a polypeptide. Physical interactions with Hsp90 should probably be regarded as weak evidence that Hsp90 modulates the function of a gene product (or vice versa), because protein–protein interactions are notoriously promiscuous *in vitro*. Exacerbating this limitation, Hsp90 displays a highly charged surface capable of binding a wide range of proteins, and Hsp90 is notoriously "sticky," in that it binds non-specifically to a wide range of inert chromatographic pull-down media. These facets of Hsp90 biochemistry place certain burdens on studies assaying binding to Hsp90 in the absence of evidence for a functional interaction. Nonethe-less, Hsp90 binding is an extremely important part of the larger puzzle, because it addresses the question of intermediary functional interactions raised above. Throughout this review, we will use the term "Hsp90-interacting" to describe both physical and functional associations with Hsp90.

An additional complication in studying interactions with Hsp90 is the challenge of determining whether a given interaction reflects Hsp90's chaperoning of a client substrate, versus an Hsp90 interaction with a protein (e.g., a co-chaperone) that regulates Hsp90 function. Traditional criteria for making this distinction appear below, elsewhere in this issue, and in the literature [9,20].

2.2. High throughput genetic screens

The first attempts to identify novel Hsp90-interacting partners were carried out by Susan Linquist's group, who screened *Saccharomyces cerevisiae* for mutations that were synthetically lethal when combined with Hsp90 mutants [21], or for genes that functioned as multi-copy suppressors of Hsp90 deficiencies [22]. The first approach screens for gene products whose function is required for cell growth or viability under conditions where Hsp90's function is compromised, while the latter approach screens for gene products whose overexpression restores the growth of cells that are Hsp90-deficient.

Extending these studies, Hsp90's functional interactions have been exhaustively mapped using synthetic genetic arrays (SGA), and chemical-genetic screens for gene deletions that create hypersensitivity to Hsp90 inhibition in yeast [16,18]. These genetic screens are based on the logic that if a gene becomes essential when the function of Hsp90 is compromised, Hsp90 may be required for the proper folding of a protein whose function overlaps with that of the deleted gene [16]. While these assays do not readily fit a classification as "proteomics," any discussion of proteomics assessments of Hsp90 function would be incomplete without them.

In the SGA approach, the Houry group utilized a haploid query strain expressing a temperature-sensitive allele of Hsp90 [16]. This strain was crossed against approximately 4000 haploid strains, each bearing a single deletion of a non-essential gene. Double-mutant haploid progeny strains were then assessed for a combinatorial synthetic growth defect, thus reporting a functional interaction between Hsp90 and the deleted gene/gene product.

In the chemical-genetic approach, both the lab of Houry and the lab of Frydman each propagated libraries of approximately 4000 viable bar-coded yeast deletion mutants in the presence of the Hsp90 inhibitors geldanamycin or macbecin II (respectively) [16,18]. After passage, DNA from surviving strains was isolated and assayed by microarray detection of the PCR-amplified gene-embedded bar codes. In this assay for deletion-induced sensitivity, a genetic interaction with Hsp90 results in the loss of the Hsp90-interacting strains from the population, with this loss being evident in loss of signal on the barcode microarray assay.

Several criteria are used to validate the "hit lists" of candidate Hsp90-interacting genes identified in these HTP genetic screens. One criterion is the re-identification of previously characterized Hsp90 co-chaperone partners and known client families or pathways, thus validating the assay's ability to identify Hsp90 interactions. A second criteria is the identification of multiple components of an individual cellular pathway or process, validating the conclusion that Hsp90 functions in that pathway. A third criteria is that results from discrete assays (performed in separate labs or using orthogonal genetic techniques) demonstrate significant overlaps in their lists of Hsp90-interacting genes, validating the individual overlapping genes as high-confidence subsets within the larger HTP data. As a fourth criterion, subsequent detailed characterizations have confirmed Hsp90's interactions with individual novel genes identified in the screen. Other more subtle bioinformatic criteria have also been presented [18].

Though similar HTP genetic techniques have not been used to assess Hsp90 function in human cells, highly specific small-molecule inhibitors of Hsp90 have proven to have similar potential. In this approach, cells are treated with Hsp90 inhibitor and subsequently analyzed using proteomics techniques, thereby identifying sets of proteins whose expression is governed by Hsp90 function. This approach is analogous to functional assays in Hsp90-deficient yeast, but assesses direct changes in protein expression rather than genetic deficiencies that compromise growth.

Several criteria are available to validate results from inhibitor-based proteomics studies of Hsp90 function. Based on well-established precedents, we should expect that an N-terminal Hsp90 inhibitor should increase the expression of Hsp70 and (to a more limited extent) Hsp90. Since both Hsp90 and Hsp70 are abundant and readily detected, this is an easily achievable validating benchmark. Similar expectations extrapolate to any other gene products regulated by HSF1, if they are apparent in the proteomics data set.

As another validator, N-terminal Hsp90 inhibitors deplete Hsp90 client proteins from treated cells. This seminal finding [10] has been duplicated for nearly every Hsp90 client protein studied in detail, and is a dogmatic hallmark of Hsp90 dependency. However, this criterion is a difficult benchmark for proteomics assays of Hsp90 function: while most Hsp90 clients are expressed at levels that are easily assayed by Western blotting, it is much more difficult to detect them using mass spectrometry. None-the-less, we might reasonably anticipate that when known Hsp90 clients are detected, they should be down-regulated by Hsp90 inhibitors that target Hsp90's N-terminus. In these cases, and those above, it is important to assess the whole dataset for these behaviors, and to avoid cherry picking data that support the study's validity while ignoring those that question it.

2.3. High throughput interaction screens

The strategies above focus on Hsp90 function, but physical interactions between proteins are often the first evidence of a functional interaction. Thus, substantial effort has been directed toward identifying proteins that bind to Hsp90. These studies often utilize affinity purification of Hsp90, subsequently using one or more mass spectrometry-based assays to identify co-purifying proteins. Hsp90 has been affinity purified using antibodies that directly bind Hsp90, and by expressing affinity-tagged Hsp90 gene constructs. This second