Heat Shock Factor 1 in Protein Homeostasis and Oncogenic Signal Integration

Trisha Home, Roy A. Jensen, and Rekha Rao

Abstract

Heat shock factor 1 (HSF1) is a stress-inducible transcription factor and has been described as a multi-faceted modulator of tumorigenesis. Heat shock, accumulation of misfolded proteins, or malignant transformation promotes the activation and nuclear translocation of HSF1, where it binds to the promoters of heat shock proteins and an array of nonheat shock-regulated proteins to upregulate their transcription. These stress-responsive and tumor-promoting genes in turn alter the ability of tumor cells to respond to a variety of stresses and enable them to thrive in less than favorable growth conditions. Although a direct role for HSF1 in promoting mRNA transcription of tumor-promoting genes has been suggested, it appears that this property is context- and cell-type dependent. Furthermore, recent studies have demonstrated a direct involvement of mTOR signaling in regulating HSF1-mediated transcription, thus establishing a direct link between protein translation and HSF1 activity. Interestingly, there is a growing understanding of the signaling pathways that are modulated by HSF1 in a variety of tumor types and the co-option of these survival pathways by HSF1 to promote tumorigenesis. This review will focus on the role of HSF1 in protein homeostasis and HSF1-mediated oncogenic signaling pathways that together promote tumorigenesis. Cancer Res; 75(6): 907–12. ©2015 AACR.

Introduction

Together with heat shock proteins (HSP), the Heat shock factor 1 (HSF1) is a major nononcogenic partner in tumorigenesis (1, 2). Tumors are addicted to several phenotypic traits that are not essential for tumor initiation, but do seem to play a critical role in its establishment and sustenance. These nononcogenic, addictive traits are collectively referred to as the "hallmarks of cancer" and include evading apoptosis, the immune system, and growth suppressors, sustaining proliferative signaling, activating invasion and metastasis, enabling replicative immortality, and inducing angiogenesis (3). These hallmarks of cancer are not surprisingly common to all types of cancers and are dependent on a complex network of cancerous and tumor-associated "normal" cells that together form the tumor microenvironment that capacitates tumorigenesis (3). More recently, a stress-associated phenotype of cancer was added to the already existing hallmarks, which is a consequence of the multitude of stressful conditions that all tumors are exposed to, including replicative, mitotic, proteotoxic, metabolic, and oxidative stress (4). Proteotoxic stress manifests as a consequence of aneuploidy, genomic instability, and the resulting nonstochiometric levels of protein subunits, in cancer cells. These factors result in the heightened dependence of cancer cells on molecular chaperones and cellular stress response mechanisms that aid in proper folding of proteins. The intricate balance between maintaining protein translation, protein folding, and sensing perturbations in protein homeostasis is key to survival of cells that experience proteotoxic stress. We begin this review with an account of the existing evidence for the role of HSF1 in maintaining protein homeostasis and go on to describe the recently uncovered, tumor-promoting functions of HSF1, as well as specific signaling pathways that it facilitates.

HSF1 and Protein Homeostasis

Maintenance of protein homeostasis is essential for the survival of all cells—both normal and cancerous. Molecular chaperones serve as the housekeepers of protein homeostasis, maintaining a perfect balance between the folding of protein polypeptides and the removal of misfolded proteins. HSP90, an ATP-dependent chaperone, and its associated cochaperones (HSP70, p23, AHA1, and CDC37) promote the folding and maturation of several HSP90 substrates also known as "client" proteins (5). Polypeptides that fail to be properly folded are targeted for degradation by the proteasomes (2). HSP70 and its cochaperone HSP40, chaperone newly synthesized proteins, or misfolded proteins induced during stress (6). Unsuccessful refolding by HSP70 directs misfolded proteins to polyubiquitylation and degradation by the proteasomes. Impairment of HSP90 chaperone function results in the polyubiquitylation and proteasomal degradation of its client proteins, such as HER2, estrogen receptors, AKT, MET, VEGFR, BRCA-1, etc. HSP90 clients are often mutated or overexpressed in cancer cells. As a result, tumors show an increased dependency on HSP90 function compared with normal cells. Consistent with this observation, a major fraction of HSP90 also exists in complex with cochaperones and exhibits higher ATPase activity in cancer cells (7). Inhibition of HSP90 function therefore not only promotes proteotoxic stress but also results in the depletion of HSP90-dependent client proteins, many of which are essential for cell survival and/or proliferation of tumors.

HSPs are regulated at the mRNA level by the stress-inducible transcription factor, HSF1. Accumulation of misfolded proteins in the cells leads to proteotoxic stress and activation of heat shock
response (HSR). In the nonstressed state, HSF1 remains inactive in the cytosol in complex (also referred to as the repressive complex) with histone deacetylase 6, heat shock protein 90, and the ATP-dependent de-aggregate, p97 (HDAC6-HSP90-p97; refs. 8, 9). Although the complex is repressive with respect to inhibiting HSF1 activity, it might in fact enhance HSP90 activity, as it brings HSP90 in close proximity to its deacetylase, HDAC6. It is well established that HSP90 is regulated by many posttranslational modifications, of which its deacetylation is important for its chaperoning activity (10, 11). Cellular stress disrupts the repressive complex, leading to dissociation of HSP90 and activation of HSF1, resulting in its trimerization, phosphorylation, and translocation to the nucleus. In the nucleus, HSF1 trimers bind to heat shock elements within the genome, and the ATP-dependent de-aggregase, p97 (HDAC6-HSP90-p97; refs. 8, 9). Although the complex is repressive with respect to inhibiting HSF1 activity, it might in fact enhance HSP90 activity, as it brings HSP90 in close proximity to its deacetylase, HDAC6. It is well established that HSP90 is regulated by many posttranslational modifications, of which its deacetylation is important for its chaperoning activity (10, 11). Cellular stress disrupts the repressive complex, leading to dissociation of HSP90 and activation of HSF1, resulting in its trimerization, phosphorylation, and translocation to the nucleus. In the nucleus, HSF1 trimers bind to heat shock elements within the promoters of several heat shock–responsive target genes, viz., HSP70, HSP40, HSP27, etc., and an increasing list of “non-heat shock” responsive target genes, including hypoxia-inducible factor (HIF) 1α, Glucose transporter 1 (GLUT-1), lactate dehydrogenase A (LDH-A), etc. (1).

A comprehensive list of all HSF1-regulated, nonheat shock response genes in metastatic breast cancer has been described in an elegant study, which strengthens the role of HSF1 in promoting tumorigenesis (1). Consequently, inhibition of HSF1 has been proposed to be an attractive strategy to restrict the growth of cancer cells (12). In addition, HSP90 inhibitors induce heat shock response (which leads to increased levels of HSPs), and promote autophagy, both of which can be cytoprotective (13, 14). These properties of HSP90 inhibitors have prompted exploration of alternative strategies to inhibit chaperones in cancer cells, which include the development of HSP70 inhibitors, the development of HSP90 inhibitors that do not induce the heat shock response, and the development of HSF1 inhibitors. Although the pursuit to develop HSF1 inhibitors is still in its infancy, much like HSP90 inhibitors, HSF1 inhibitors are likely to inhibit multiple facets of tumorigenesis, including metabolism, angiogenesis, invasiveness, and metastasis (1, 15).

How Might HSF1 Affect HSP90 Activity?

Although the transcriptional regulation of Hsps by HSF1 is well established, it is presently unclear whether the activity of HSP90 is influenced by the levels and activity of HSF1. One might argue that the deacetylation and functional activation of HSP90 is likely brought about by the proximity of HDAC6, an Hsp90 deacetylase, in the repressive complex (10). Consequently, nonstoichiometric levels of one or more of the individual components of the repressive complex might adversely affect HSP90 activity. However, no studies have reported the effect of HSF1 inhibition on the disassembly of the repressive complex and the its impact on HSP90 function, either directly by acetylation or other posttranslational modifications of HSP90. Experimental evidence suggests that loss of HSF1 results in the decreased stability of HSP90 client proteins (mostly kinases) and inhibits their downstream signaling (described in the more detail in the following sections; refs. 16, 17).

HSF1 and Protein Translation

The regulation of eukaryotic protein translation is a dynamic stress-responsive process that is regulated by mTOR complex (mTORC; ref. 18). mTORC1 is a rapamycin-sensitive complex composed of six proteins that senses a variety of stress signals, including protein misfolding, growth factor deprivation, energy deficiency, oxygen deficiency, and amino acid deprivation, and promotes cellular anabolism and autophagy. mTORC2 is relatively less sensitive to rapamycin and regulates cell survival, cell size, cytoskeletal organization as well as cellular metabolism. Growth factor signaling and energy-replete conditions activate mTORC1 signaling, which promotes protein and lipid biosynthesis and inhibits autophagy. mTORC1 activity also increases ribosomal biogenesis by stimulating the transcription of ribosomal RNA. Together with its upstream regulatory components including PI3K, PTEN, and AKT, mutations in mTOR signaling along with p53 mutations are the most commonly occurring genetic lesions reported in the cancer genome (18).

Several lines of evidence suggest that the mTOR pathway is intricately linked to HSF1 activity. The most direct role of mTOR in regulating HSF1 activity is through its ability to phosphorylate HSF1 at S326 following exposure to stress. HSF1 has been shown to possess several potential phosphorylation sites (S230, S303, S307, S326, etc.), of which S326 is one of the residues that is important for rendering it transactivation-competent (19). mTORC1 has been shown to possess the kinase activity responsible for HSF1 phosphorylation and nuclear translocation (19).

The evidence for the involvement of protein translation machinery in the transcriptional activation of HSF1-dependent mRNAs comes from a study in yeast that identified perturbations that induce HSF1 promoter activity. The primary screen was the activation of a GFP reporter driven by HSF1 promoter that was translated in a genome-wide loss-of-function library in yeast. (20). Consistent with the role of HSF1 in alleviating proteotoxic stress, stressors that induced HSF1 promoter activity were the deletion of chaperones and components of the proteasome. Other genes that induced HSF1 activity were chromatin modifiers, positive regulators of protein kinase A activity, and components of the ribosome quality control complex (RQC). The RQC is a protein complex comprising RQC1, Listerin (LTN1), CDC48 (a p97 ortholog), TAE2 (a protein translation-associated element), etc. that degrades aberrantly translated, stalled proteins on the ribosomes (20, 21). This study established the role of TAE2 in activating HSF1 in response to the accumulation of aberrantly translated proteins with a polyhistidine tract that arise from the translation of mRNA poly(A) tails. Although the specific nature of signaling events that direct the activation of HSF1 remains elusive, this study links mTOR to the activation of HSF1 in response to proteotoxic stress. A subsequent study fortified the role of mTOR in HSF1 activation in which a luciferase-tagged HSF1 reporter construct was used to screen for agents that inhibited HSF1 promoter activity in response to proteotoxic stress. The chemical screen identified that the most potent inhibitor of HSF1 activity was an inhibitor of the eukaryotic translation initiation factor 4A (eIF4A), called rocaglamide (21, 22). The study also demonstrated that translation inhibition and HSF1 inactivation are accompanied by decreased dependence of cancer cells on glucose uptake and rewiring of cellular metabolism to promote anabolism. Consistent with the role of mTOR signaling in HSF1-mediated transcription, inhibition of the mTOR pathway abrogates nuclear translocation of HSF1 and the induction of HSPs following exposure to Hsp90 inhibitors. (23). Taken together, these studies provide ample evidence to suggest that HSF1-dependent transactivation is regulated by mTOR activity. It remains to seen whether mTOR activity regulates the translation of specific factors.
HSF1 in Tumorigenesis

Figure 1 is a pictorial representation of the frequency of genetic mutations (4% or above), including point mutations, amplifications, deletion and/or copy number variations observed for HSF1 across a variety of tumors. It is readily apparent that HSF1 is highly amplified in a variety of tumors and tumor cell lines with up to 30% frequency of alteration in some of the most aggressive tumors (data analyzed using www.cbioportal.org). Although much of information about HSF1 reported in The Cancer Genome Atlas (TCGA) comes from sequencing or analysis of solid tumors, there is evidence to suggest that HSF1 might indeed be highly expressed or activated in certain types of hematologic malignancies, including multiple myeloma and chronic lymphocytic leukemia (R. Rao, unpublished data; refs. 24, 25). Given the role of HSF1 in adaptive tolerance of cells to a variety of stresses, including malignant transformation, it is not surprising that cancer genomes have evolved to co-opt HSF1 to their advantage. Recent evidence suggests that HSF1 activation in cancer-associated fibroblasts (CAF) or the tumor microenvironment alters tumor gene expression and is associated with poor patient outcome. Interestingly, HSF1 in the stromal cells activates genes involved in development, proliferation, and response to wound healing, with TGFβ and SDF1 being the top activated stromal genes. Thus, it appears as if tumor cells hijack cell autonomous and noncell autonomous HSF1-dependent pathways to promote their own survival (26).

Figure 1.
A cross-cancer alteration summary for HSF1. Mutational status for HSF1 with 4% or higher alteration frequency in a variety of tumors and tumor cell lines. Most data reported are deposited at TCGA (provisional) or have been published (TCGA published). 1, ovarian; 2, ovarian (TCGA); 3, NCI-60; 4, prostate (38); 5, liver; 6, CCLE; 7, breast; 8, uterine carcinoma; 9, head and neck; 10, head and neck (TCGA published); 11, lung adenocarcinoma; 12, bladder; 13, breast (TCGA published); 14, melanoma; 15, prostate; 16, bladder (TCGA published); 17, pancreas; 18, stomach; 19, uterine; 20, uterine (TCGA published); 21, glioma; 22, colorectal; 23, lung adenocarcinoma (TCGA published); 24, adenoid cystic carcinoma; 25, prostate (39); 26, colorectal (TCGA published), CCLE, Cancer Cell Line Encyclopedia; NCI-60, National Cancer Institute Cancer Cell line panel.
hyperactivation of the RAS/MAPK signaling pathway resulting in neurofibromatosis type 1, an inherited form of cancer. These studies point to the fact that signaling events impinging on the MAPK signaling is a recurring theme in the functional activation of HSF1.

HSF1 and human epidermal growth factor receptor-2 signaling in breast cancer

HER2/Neu belongs to the EGFR tyrosine kinase family and is amplified/overexpressed in human and ovarian cancers (29). Human epidermal growth factor receptor-2 (HER2) signals through the PI3K–AKT and the Ras–Raf–MEK–ERK pathways to promote tumor proliferation. Several studies have implicated the role of Hsf1 in HER2-induced tumorigenesis and metastasis of mammary tumors. HER2 overexpression in breast cancer cells results in increased activation of HSF1 and its downstream target LDH-A, a known regulator of glycolysis (30). Meng and colleagues (31) demonstrated that mammary xenografts with Hsf1 knockdown are unable to form tumors in vivo because of the induction of senescence and the upregulation of the cell-cycle inhibitor p21. In a subsequent study, the role of Hsf1 in Her2-induced mammary tumors was studied in a genetic model of mouse mammary tumorigenesis utilizing MMTV-Her2 mice with Hsf1 knockout mice. As expected, compared with MMTV-Her2/Neu mice, Hsf1+/− Neu− mice developed mammary tumors at a very low frequency with longer latency periods and showed very poor propensity to metastasize. This was accompanied by enhanced levels of E-cadherin and β-catenin staining in Hsf1+/− Neu− mice compared...
with Hsf1+/+/Neu− mice, suggesting that loss of Hsf1 is correlated with the loss of mesenchymal markers and a reduced epithelial to mesenchymal transition (and hence reduced metastasis). Hsf1+/+/Neu− mice also showed reduced association of the Hsp90 client protein c-RAF with HSP90 and reduced levels of AKT-ERK1/2 signaling (16). Similar findings with respect to stabilization of Hsp90 client proteins by Her2/Hsf1 axis came from a study where inhibition of Her2 signaling resulted in the inhibition of Hsf1 activity (phosphorylation) and the destabilization of Hsp90 clients including MIF (macrophage migration inhibitory factor) and AKT (17). Recent studies have demonstrated that overexpression of mutant p53 also activates EGFR/Her2, leading to enhanced PI3K and MAPK signaling, which results in the phosphorylation and activation of Hsf1 (32). These studies suggest that Hsf1-dependent chaperoning of Hsp90 client proteins is dependent on growth factor (growth factor–PK3K–AKT) signaling. Furthermore, high expression and nuclear localization of Hsf1 confer poor prognosis in estrogen receptor–positive (ER+) breast tumors as well, which suggests that Hsf1 activation is not a distinguishing feature of HER2-overexpressing breast tumors (33). Indeed, Hsf1-dependent gene expression in cancer is conserved across many tumor types and is strongly associated with metastasis and death in at least breast, colon, endometrial, and lung cancer (1, 34).

**HSF1 and the protein kinase C 0**

Protein kinase C 0 (PKC0) is a novel isoform of the protein kinase C family of proteins that catalyzes the inhibitory phosphorylation insulin receptor substrate 1 (IRS1). This results in reduced phosphorylation of AKT, leading to reduced glucose uptake or glucose intolerance. PKC0 activation also leads to the phosphorylation of Hsf1 at Ser333, resulting in its activation and enforced dependence of tumor cells to glucose. Simultaneous activation of Hsf1 activation and PKC0 in cancer cells that express this isoform of PKC, such as kidney tumors, results in synthetic lethality, as decreased glucose uptake and enhanced dependence on glucose would starve the cancer cells to death (35). Thus, in some scenarios, Hsf1 activation is not permissive to tumorigenesis.

**Regulation of HuR-dependent genes by HSF1**

HuR is an Hsf1-regulated gene that controls tumor neovascularization, metabolism, invasion, and metastasis. Studies utilizing MMTV Neu mice and Hsf1+/−/Neu mice revealed that loss of Hsf1 results in reduced mammary duct branching, an absence of alveolar branching, and reduced tumor blood vessel density. Tumor xenographs from Hsf1 knockout cells also showed reduced levels of HIF1α and its downstream targets by inhibiting HIF1α translation. The same study also revealed that Hsf1 knockdown leads to reduced transcription of the RNA binding protein HuR, which in turn regulates the mRNA stability or translation of many targets including HIF1α (36). Another example of an HuR-regulated gene that is downregulated following knockdown of Hsf1 in CD44+/CD24− breast cancer stem cells is β-catenin. In addition, Hsf1 knockdown increased the expression of lincRNA-p21, a long noncoding RNA, that inhibits β-catenin translation (37). The observed differences could stem from the fact that β-catenin induction is an Her2-inducible event in breast cancer. Taken together, these observations suggest that Hsf1-mediated effects could be a result of the transcriptional regulation of factors (e.g., HuR or other noncoding RNAs) that in turn regulate the stability or translation of their mRNA targets, in a context-dependent manner.

**Concluding Remarks**

In conclusion, Hsf1 can affect nodal points in oncogenic signaling by one or more of the following mechanisms: (i) By the transcriptional activation of signaling proteins and regulators of mRNA translation, (ii) by Hsp90-dependent chaperoning of tumor-promoting genes, (iii) by signal integration and amplification by the oncogenic pathways, including the RAS–RAF–MAPK or the PI3K–Akt–mTOR pathway, and (iv) by the activation of tumor-promoting signaling pathways in the stromal cells (Fig. 2). Considering the multitude of ways in which Hsf1 might promote tumorigenesis, it is reasonable to conclude that Hsf1 is generally permissive to tumorigenesis. Therefore, targeting Hsf1 would be most beneficial in tumors that are addicted to signaling pathways that work in concert with Hsf1.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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