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HSF1: Guardian of proteostasis in cancer

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Abstract

Proteomic instability is causally related to human diseases. In guarding proteome stability, the heat shock factor 1 (HSF1)-mediated proteotoxic stress response plays a pivotal role. Contrasting with its beneficial role of enhancing cell survival, recent findings have revealed a compelling pro-oncogenic role for HSF1. However, the mechanisms underlying the persistent activation and function of HSF1 within malignancy remain poorly understood. Emerging evidence reveals that oncogenic signaling mobilizes HSF1 and that cancer cells rely on HSF1 to avert proteomic instability and repress tumor-suppressive amyloidogenesis. In aggregate, these new developments suggest that cancer cells endure chronic proteotoxic stress and that proteomic instability is intrinsically associated with malignant state, a characteristic that could be exploited to combat cancer.

Keywords

HSF1; proteotoxic stress; proteome homeostasis; amyloidogenesis; tumor suppression

Proteotoxic stress: an emerging characteristic of cancer

Malignant transformation brings forth profound alterations in a wide array of biological processes, inevitably disturbing intricate cellular homeostatic states. In cancerous cells, not surprisingly, various types of biological stress (see Glossary) accompany their malignant behavior, among which genotoxic [1,2], oxidative [3,4], and metabolic stress [5,6] are well documented and widely recognized. In contrast, little has been known about proteotoxic stress in cancer, despite its prominent manifestation in human neurodegenerative disorders [7]. However, emerging evidence reveals proteotoxic stress as a widespread feature in cancer, and is beginning to illuminate its previously unappreciated impact on oncogenesis.

Proteostasis (or proteome homeostasis) [8], a process by which cells balance the processes of protein biosynthesis, folding, and degradation, is vital to cellular fitness. However, a variety of environmental cues constantly challenge this homeostatic state, disruption of which, elicits proteotoxic stress in cells [8]. Given the necessity of a healthy proteome, the

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cytoprotective mechanism named the heat-shock, or proteotoxic stress, response (PSR) has evolved to counter such stress [9,10], and is characterized by the induction of heat-shock proteins (HSPs) [9,10]. HSPs are molecular chaperones that maintain cellular proteostasis through facilitation of folding, transportation, ubiquitination, and proteasomal degradation of proteins [11–13]. A small group of transcription factors named heat-shock factors (HSFs) specialize in activating the PSR in the face of proteotoxic stress (Box 1) [14,15]. Among the many HSFs, HSF1 is the master regulator of this transcriptional program in mammals [9,16]. Indeed, genetic ablation of *Hsf1* in mice abrogates HSP induction, rendering cells vulnerable to proteotoxic stress [17–19].

Box 1

HSF paralogs, the proteotoxic stress response, and development

While yeast and invertebrates express a single HSF, at least nine HSF paralogs HSF1, 2, 3, 4, 5, X1, X2, Y1, and Y2 have been identified in vertebrates [15]. All HSF proteins share several structural homologies, including the N-terminal helix-turn-helix DNA-binding domain (DBD), the coiled-coil trimerization domain enriched for hydrophobic heptad repeats (HR), and the C-terminal transactivation domain (AD) [15,77]. HSFs bind *via* their DBD to consensus Heat Shock Elements (HSE) that canonically comprise adjacent inverted arrays of a specific sequence motif (5'-nGAAn-3') [15,77]. Whereas mammalian cells deficient for *Hsf2*, *Hsf3*, or *Hsf4* still retain stress-induced expression of *Hsp* genes in mice [100–102], *Hsf1* ablation abrogates this response [17–19], indicating its essentiality to the PSR. Nonetheless, accumulating evidence suggests that other HSFs could modify the transcriptional program mediated by HSF1. For example, through heterotrimerization with HSF1, HSF2 either enhances *Hsp72* expression or represses expression of *Hsp40* and *Hsp110* [103]. Moreover, HSF3 is able to induce some non-*Hsp* genes that are also regulated by HSF1 and, importantly, protect *Hsf1*-deficient cells from proteotoxic stress [101]. In addition to their roles in the PSR, HSFs play important roles during development, as revealed in genetically engineered mouse models. Deletion of *Hsf1* in mice causes placental defects, prenatal lethality, and female infertility [17]; *Hsf2*-deficient mice display enlarged brain ventricles and defective gametogenesis [104]; and mice deficient for *Hsf4* develop cataracts [102]. In contrast, the biological functions of HSF3, HSF5, and sex chromosome-linked HSFY1, HSFY2, HSFY1, and HSFY2 remain largely unclear.

Acute proteotoxic stress translocates HSF1 from the cytosol to the nucleus where it promotes increased transcription of genes involved in protein quality control, thereby allowing cells to survive proteotoxic stress [17–19] (Figure 1). The activation of HSF1 is transient and attenuates in parallel with the alleviation of stress [20]. Moreover, HSF1 is dispensable for cellular and organismal viability under normal non-stressed conditions [17–19,21]. In contrast, HSF1 appears to remain constitutively active in cancer cells [22, 23], suggesting the presence of chronic proteotoxic stress. Indeed, the expression of HSPs is notably elevated in a large number of human cancers [24,25]. Hence, malignancy epitomizes a pathological state inflicted with chronic proteotoxic stress.

Recent evidence is beginning to unravel the molecular mechanisms of HSF1 activation and function in regulating proteostasis in cancer. It is conceivable that the hostile tumor microenvironment, often acidic and hypoxic [26,27], is disruptive to proteostasis in cancer and stress-provoking. In addition, proteotoxic stress could arise cell-autonomously due to cell-intrinsic alterations. For example, protein biosynthesis is markedly enhanced in cancer cells due to hyperactivation of mTORC1 [28,29], a key regulator of translation [30,31]. Genomic instability of cancer cells also exacerbates proteostasis imbalance. Aneuploidy can increase protein dosage, subsequently exaggerating the proteomic burden [32,33]. Moreover, oxidative damage of proteins is augmented due to elevated levels of reactive oxygen species (ROS) in cancer cells [34,35]. Also, numerous genetic mutations cause protein conformational changes that often lead to decreased protein stability [36].

In this review, we summarize the recent exciting findings in proteomic instability and underscore the critical role of HSF1 and its mediated PSR in preserving proteostasis in cancer. Moreover, we highlight cancer fragile proteostasis as a potential therapeutic target as well as a novel biomarker.

Regulation of HSF1 activity through phosphorylation

The activation of HSF1 upon challenge by proteotoxic stressors is reliant on its phosphorylation (Figure 1). Several phosphorylation events have been reported to promote HSF1 activation, including Ser230 phosphorylation by calcium/calmodulin-dependent protein kinase II (CaMKII) [37], Ser320 phosphorylation by protein kinase A (PKA) [38], Thr142 phosphorylation by casein kinase 2 (CK2) [39], and Ser419 phosphorylation by polo-like kinase 1 (PLK1) [40]. Furthermore, phosphorylation of Ser326 on HSF1 was identified as a modification that is critical to stress-induced HSF1 activation [41]. Originally this modification was found to be mediated by mTOR [42]; however, a new study indicates that the RAS/MAPK signaling pathway also regulates HSF1 activation through Ser326 phosphorylation [43] (Figure 2). Moreover, some HSF1-phosphorylating events negatively impact its transcriptional activity such as Ser121 phosphorylation, which has been linked to metabolic sensors, Ser303, Ser307, and Ser363 phosphorylation [44–46].

Activation of HSF1 by oncogenic RAS signaling

The canonical RAS/MAPK signaling pathway governs a plethora of cellular processes including proliferation, differentiation, transcription and translation, and cell survival [47,48], and anomalies of this signaling pathway are causally related to a number of human pathological conditions, collectively named RASopathies [49]. Notably, it has been estimated that approximately 30% of all human cancers possess activating somatic mutations in components of this signaling cascade, including *RAS*, *RAF*, and *MEK* genes [50]. Germline mutations of this signaling pathway also underlie several hereditary diseases, including Neurofibromatosis type I (NF1) [51], Costello syndrome (CS) [52], Noonan syndrome (NS) [53], and Leopard syndrome (LS) [54].

In a recent study, MEK blockade markedly impaired HSF1 Ser326 phosphorylation induced by heat stress, while ERK blockade heightened this phosphorylation [43]. Since ERK has long been regarded as the ultimate effector of the RAS/MAPK signaling cascade [47,48], it

would be expected that ERK would mediate HSF1 Ser326 phosphorylation. Instead, MEK, the immediate upstream kinase of ERK, physically interacts with and phosphorylates HSF1 at Ser326, both *in vivo* and *in vitro* [43]. Furthermore, under heat stress ERK, MEK, and HSF1 assemble into a ternary protein complex, wherein ERK suppresses HSF1 Ser326 phosphorylation through inhibitory phosphorylation of MEK at Thr292 and Thr386 [43] (Figure 2). Congruent with its role as a negative regulator of the RAS oncoprotein, loss of the tumor suppressor *NF1* constitutively mobilizes HSF1 through activation of MEK [22].

This molecular model reconciles the opposing effects of MEK and ERK on HSF1 activation. The finding that HSF1, in parallel to ERK, is another physiological substrate for MEK is significant in that it has long been thought that ERK was the only substrate for MEK; this finding thus reveals a previously unappreciated complexity of RAS/MAPK signaling. Further, this finding not only highlights a new biological function of RAS/MAPK signaling in regulating the PSR, but also shifts the canonical paradigm of the RAS/MAPK signaling cascade. That is, MEK acts as the central nexus of this signaling cascade, conveying RAS activity to both ERK- and HSF1-mediated pathways simultaneously (Figure 2). While the ERK- and HSF1-mediated pathways are biologically distinct, they are intimately interconnected. ERK, in a negative feedback mechanism, finely attunes MEK-mediated HSF1 activation. These complex regulatory configurations, while ensuring a tight coordination between ERK- and HSF1-mediated pathways, also provide a means to heighten HSF1 activation through ERK inhibition. Given the widespread aberrant alterations in the RAS/RAF/MEK signaling cascade in human malignancies, these new findings reveal that constitutive activation of HSF1 and its mediated PSR is deeply rooted within oncogenic process.

Suppression of HSF1 by metabolic-stress signaling

A recent study reveals that metabolic stressors, including nutrient deprivation and metformin, suppress transcriptional activation of HSF1 in large part through AMP-activated protein kinase (AMPK) [55] (Figure 2). AMPK, acting as a key metabolic sensor, closely gauges intracellular AMP/ATP or ADP/ATP ratios [56]. Upon activation under a low cellular energy state, AMPK phosphorylates numerous effectors that control diverse biological processes, including lipogenesis, gluconeogenesis, autophagy, glycolysis, fatty acid oxidation, and protein synthesis [57]. This systemic cellular reaction is collectively referred to as the metabolic stress response (MSR). Through enhancement of ATP production and reduction of ATP consumption, the AMPK-mediated MSR plays a pivotal role in antagonizing metabolic stress and reinstating cellular energy homeostasis [56,57]. Of note, liver kinase B1 (LKB1/STK11), an immediate upstream kinase of AMPK [58], is a known tumor suppressor. Germline loss-of-function mutations of *LKB1* have been causally linked to Peutz-Jeghers syndrome (PJS) in humans, a cancer-predisposition disorder that manifests hamartomatous polyps in the gastrointestinal tract and mucocutaneous pigmentation [59].

Upon activation by metabolic stress, AMPK physically interacts with and phosphorylates HSF1 at Ser121 [55] (Figure 2). This phosphorylation impairs HSF1 activation, in part, through impedance of HSF1 nuclear translocation [55]. Congruently, glucose deprivation

and metformin treatment, both of which provoke metabolic stress and activate AMPK, impair the HSF1-mediated PSR and, in turn, render cells susceptible to heat stress [55]. Importantly, both glucose deprivation and metformin treatment also suppress constitutive HSF1 activation within human cancer cells, depleting cellular chaperoning capacity and subsequently destabilizing the cancer proteome [55]. The identification of HSF1 as a new physiological substrate for AMPK highlights a previously unrecognized relationship between the metabolic and proteotoxic stress responses. In addition to revealing a new mechanism of action underlying the emergent anti-neoplastic effects of metformin, these findings also suggest that activation of HSF1 and suppression of proteotoxic stress may be an important outcome of a cancer cell's reliance on glucose, a phenomenon known as "the Warburg effect".

Following metabolic stress, activated AMPK also suppresses mTORC1 through phosphorylation of RAPTOR [60]. Thus, metabolic stress could also inactivate HSF1 through mTORC1 inhibition, given the reported HSF1 regulation by mTORC1 [42].

HSF1: a powerful multifaceted facilitator of oncogenesis

In line with its constitutive activation in cancer, amassing evidence has demonstrated that HSF1 potently facilitates oncogenesis. The first proof came from two independent *in vivo* studies using genetically engineered mouse models. In these experiments, genetic deletion of *Hsf1* in mice impaired lymphomagenesis due to *Trp53* deficiency [61], chemical-induced skin carcinogenesis [21], as well as the multiple instances of tumorigenesis initiated by a "hot-spot" *Trp53* mutation [21]. Subsequently, it was shown that *Hsf1* deficiency suppresses the development of hepatocellular carcinomas (HCC) induced by pro-carcinogen diethylnitrosamine (DEN) [62], delays the onset and lung metastasis of mammary tumors in MMTV-HER2/Neu transgenic mice [63,64], and impairs carcinogenesis associated with loss of *Nf1* [22].

The pro-oncogenic effects of HSF1 have been further demonstrated in xenograft mouse models. *In vivo*, *HSF1* depletion by RNA interference suppresses growth of human mammary epithelial cells overexpressing HER2 [65], impairs growth, invasion, and metastasis of HCC cells [66,67], and antagonizes growth, invasion, and metastasis of human melanoma cells [68,69]. Conversely, enhanced HSF1 expression promotes *in vivo* growth, invasion, and metastasis of melanoma cells [43,70,71]. In aggregate, these findings pinpoint HSF1 as a potent pro-oncogenic factor functioning in diverse malignancies.

In addition to its critical role in tumor initiation, emerging evidence strongly suggests that cancer cells become reliant on HSF1 to maintain their malignant phenotypes. Lentiviral shRNA-mediated *HSF1* depletion markedly impairs the growth and survival of a collection of human cancer cell lines that are derived from diverse tissue origins and harbor a variety of genetic abnormalities [21]. Independent studies further show that *HSF1* depletion or inhibition impairs proliferation of human melanoma cells and HCC cells [43,68,69], induces apoptosis in multiple myeloma cells [72], and compromises viabilities of malignant peripheral nerve sheath tumor (MPNST) cells [22], pancreaticobiliary cancer cells [73], and oral squamous cell carcinoma cells (OSCC) [74].

In sharp contrast, *HSF1* depletion barely affects non-transformed cells [21, 73]. In accordance with this result, *Hsf1*-deficient primary cells and mice remain viable under normal growth conditions [17,18,21]. The dependence of malignant cells on HSF1, at least in part, reflects their intrinsic state of chronic stress, an exemplification of the “non-oncogene addiction” phenomenon of cancer [75]. Importantly, the addiction to HSF1 imparts an inherent vulnerability of cancer that could be exploited for effective anti-cancer therapies.

HSF1 guards the cancer proteome and suppresses amyloidogenesis

It has been widely recognized that *HSP* genes are the classic transcriptional targets of HSF1. As molecular chaperones, HSPs maintain the functional conformations and stability of an immense number of cellular proteins, many of which are key oncoproteins. Just a few examples from an ever-increasing list of HSP's client proteins include ERBB2/HER2, c-MET, CYCLIN D1, CDK4, BRAF, and AKT [76,77]. It is noteworthy that the stabilities of mutant driver oncoproteins generated *de novo* in cancer, including BCR-ABL, EML4-ALK, and mutant TP53, are particularly reliant on HSPs [76,78]. In line with its role in stabilizing the cancer proteome, HSF1 depletion diminishes oncoproteins in cancer cells, including EGFR, mutant TP53, KSR1, AKT, and BRAF [22,68,79]. Of particular interest, HSF1 depletion also destabilizes ribosomal subunit proteins, including RPL13 and RPL17 [43]. This finding uncovers a previously unrecognized impact of HSF1 on ribosome machinery and reveals an intimate link between cellular chaperoning and translational capacity. Thus, HSF1 promotes oncogenesis not only through enhancement of general protein synthesis but also through stabilization of oncoproteins.

Beyond shortening the half-lives of proteins, *HSF1* depletion elicits global protein aggregation as evidenced by increased levels of ubiquitinated proteins that become resistant to detergent extraction [43, 55]. Even more strikingly, amyloids, protein aggregates that are enriched for β sheet structures and are causally associated with several neurodegenerative disorders in humans, emerge in *HSF1*-deficient cancer cells [43]. Congruent with the critical role of MEK in activating HSF1, pharmacological inhibition of MEK, similarly, induces protein aggregation and amyloidogenesis in cancer cells [43]. Interestingly, under basal conditions in cancer cells, amyloids appear to be readily cleared by proteasomes, as combinatorial proteasome inhibition markedly enhances amyloid formation induced by MEK blockade [43]. Of great importance, malignant cells are particularly susceptible to amyloidogenesis, as the same combinatorial inhibition fails to induce amyloids in primary non-transformed cells and tissues [43]. This unique vulnerability of cancerous cells to proteomic perturbations is in accordance with their intrinsic elevated levels of proteotoxic stress.

Amyloidogenesis is tumor-suppressive as impairment of amyloidogenesis, by either amyloid-binding dyes or a neutralizing antibody, markedly antagonizes the inhibition of growth and survival of cancer cells imposed by combined MEK and proteasome inhibition [43]. Moreover, blockade of amyloid induction through *in vivo* administration of the popular amyloid stain Congo red not only accelerates melanoma growth, but also mitigates the tumor-suppressive effects of combinatorial MEK and proteasome inhibition [43].

These findings *en masse* set forth a paradigm wherein HSF1 critically guards cancer proteome homeostasis, through enhancement of protein synthesis, stabilization of oncoproteins, and suppression of tumor-suppressive protein aggregation and amyloidogenesis. Thereby, HSF1 enables robust oncogenesis (Figure 3).

Stress adaptation of cancer enabled by HSF1

The transcriptional action of HSF1 has broader implications than previously expected, extending far beyond those simply engaging in protein folding. It is now recognized that under heat stress and in cancer cells, HSF1 governs the expression of both *HSP* genes and numerous non-*HSP* genes [80]. However, HSF1-mediated transcriptional responses are distinct between cancer cells and cell exposed to heat stress, resulting in discrete genome-binding patterns of HSF1 [23], likely because the stresses endured by cancer cells differ both in type and intensity from those of the classic heat shock response. Indeed, Chromatin Immunoprecipitation Sequencing (ChIP-seq) experiments in malignant cells revealed that HSF1 regulates the expression of genes that are implicated in a diversity of biological processes, ranging from protein translation (e.g. EIF4A2 and RPL22), to cell cycle progression (e.g. CDC6, KNTC1, and POLA2), to DNA repair and chromatin remodeling (e.g. MLH1 and CBX3), to energy metabolism (e.g. FASN and PGK1), and to mRNA processing (e.g. HNRNPA3 and RBM23) [23]. These new findings suggest that through these direct transcriptional regulations, HSF1 is capable of reshaping cellular physiology at the system level by impacting a wide array of cellular pathways. Thus, in addition to guarding the cancer proteome, HSF1 fosters prolific adaptation to chronic proteotoxic stress withstood by cancer cells (Figure 3).

Discrete pro-oncogenic functions of HSF1

Beyond its systemic impacts, numerous studies have also revealed discrete functions of HSF1 that promote oncogenesis. In response to oncogenic signals, HSF1 enhances cell growth and survival. Compared to their wild-type counterparts, *Hsf1*^{−/−} mouse embryonic fibroblasts (MEFs) are refractory to marked proliferation stimulated by oncogenic RAS and platelet-derived growth factor B (PDGF-B), but exhibit heightened cell death upon expression of c-MYC and SV40 large T antigen [21]. In addition, HSF1 suppresses cellular senescence triggered by the *HER2/NEU* oncogene through induction of HSPs [65].

HSF1 also augments key oncogenic signaling. Interestingly, while RAS signaling directly activates HSF1 via MEK, this activation, in turn, enhances oncogenic RAS signaling through HSP90-mediated KSR1 stabilization [22], thus constituting a feed-forward loop. KSR1 is a key scaffold protein that is required to assemble RAF-MEK-ERK signaling complexes [81].

Furthermore, HSF1 co-opts cellular metabolism to facilitate malignant transformation. HSF1 has been shown to maintain mTORC1 activity in non-transformed cells [21]. It is widely recognized that mTORC1 plays a critical role in cancer by promoting protein translation and suppressing autophagy [28,29]. Interestingly, compared to their wild-type counterparts, *Hsf1*^{−/−} MEFs not only display impaired mTORC1 signaling but also are more sensitive to cell cycle arrest induced by the specific mTOR inhibitor rapamycin [21]. Congruent with

impaired mTORC1 activity, *Hsf1*^{-/-} MEFs are 20% smaller in cell size [21]. Although the molecular mechanisms through which HSF1 regulates mTORC1 have not been fully elucidated, this effect of HSF1 is, at least in part, mediated through HSP90, which is required for appropriate mTORC1 assembly [82]. In addition to protein synthesis, HSF1 enhances cellular uptake of glucose, an essential fuel for rampant cancer cell growth [83], in non-transformed cells [21]. HSF1 achieves this, at least in part, via suppressing expression of thioredoxin-interacting protein (TXNIP) [84], a potent suppressor of glucose uptake through regulation of GLUT1 [85]. Intriguingly, HSF1 has also been reported to stimulate lipid synthesis through suppression of insulin and AMPK signaling in the normal liver [62]. Given that elevated lipogenesis is widespread in human cancers and critical to membrane synthesis necessary for rapid cancer cell proliferation [86], this lipogenic effect of HSF1 is proposed to promote development of hepatocellular carcinomas [62].

Moreover, HSF1 is important to tumor progression. HSF1 has been reported to enhance cell migration and epithelial-mesenchymal transition (EMT) [63,64,87]. In *Hsf1*^{+/+} mouse mammary epithelial cells that express the *HER2/NEU* oncogene, TGF β stimulation induces higher levels of ERK activity and EMT, indicated by reduced expression of the epithelial marker E-Cadherin and increased expression of mesenchymal markers including SLUG and Vimentin [64]. This pro-migratory effect of HSF1 is congruent with its role in promoting tumor invasion and metastasis [64,66,70]. In addition, HSF1 is able to sustain angiogenesis in *HER2*-driven mouse mammary tumors through translational regulation of hypoxia-inducible factor 1 (HIF-1) expression [63], and support anchorage-independent growth of human multiple myeloma cells through induction of HSPs [72].

Collectively, these findings reinforce the notion that HSF1 adeptly orchestrates an extensive network of cellular functions to facilitate robust oncogenesis systemically.

Targeting fragile proteostasis in cancer: therapeutic strategies and biomarker potential

In light of its multifaceted roles in oncogenesis, it is not surprising that HSF1 is being considered as an attractive therapeutic target. Thus far, several small molecules, including quercetin [88], KNK437 [89], triptolide [90], KRIBB11 [91], and rocaglates [92] have been reported to suppress the transcriptional activity of HSF1, although the specificity of these inhibitors towards HSF1 remains to be more clearly defined. Alternatively, *HSF1*-targeting RNAi has been shown to be effective in suppressing its transcriptional activity, at least *in vitro* [21,22,68,69]. Recently, a RNA aptamer that potently blocks DNA binding of HSF1 has also been developed [93].

In addition to targeting HSF1 itself, it is feasible to suppress HSF1 activity through modulation of signaling pathways that play critical roles in regulating HSF1 activation. For example, AMPK activators or MEK inhibitors are capable of incapacitating HSF1, albeit in an indirect manner [43,55]. This promiscuity in action may prove to be advantageous with regard to eradicating malignancy, as these agents impact a wide range of pathways beyond HSF1 and thereby elicit broader and more potent therapeutic effects. While targeting HSF1 or other factors alone could suffice to destabilize the cancer proteome to some extent, it is

likely that combinatorial blockade of the proteasome will exhibit profound synergistic effects [43], and this combination may have additional merits in averting development of resistance to individual inhibitors.

Beyond being an attractive therapeutic target, HSF1 may also serve as a biomarker for cancer prognosis, a notion supported by several recent studies. A study surveying a large cohort of over 1,800 breast cancer patients reveals that in normal mammary epithelial cells HSF1 expression remains low and mainly cytoplasmic; in contrast, HSF1 expression is predominantly nuclear in the majority of malignant tissues, indicative of its activation [94]. Indeed, high levels of nuclear HSF1 proteins correlate significantly with poor prognosis among ER⁺ [94], HER2⁺ and triple-negative breast cancer patients [23]. Elevated nuclear HSF1 expression has also been observed in cervical cancer, colon cancer, lung cancer, pancreatic cancer, prostate cancer, and meningioma [23]. In addition, nuclear HSF1 expression is associated with tumor size in OSCC [95], and with metastasis and poor survival in endometrial cancer [96]. Further characterization of HSF1 activation in cancer was performed using high-throughput ChIP-seq technologies that profiled targeted genes of HSF1 in a broad range of human cancer cell lines and specimens [23]. The results yielded an “HSF1-cancer signature”, encompassing a collection of 456 HSF1-bound genes that displayed a remarkable correlation with shortened survival in patients afflicted with breast, lung, and colon cancer [23]. In aggregate, these findings strongly suggest HSF1 and its mediated stress response is a valuable prognostic marker for a wide array of human cancers. The increased HSF1 expression and activation is, likely, reflective of exacerbated intrinsic stress that inevitably arises from malignant progression.

Concluding remarks

It has become increasingly apparent that cancer cells are constantly confronted by proteotoxic stress from intrinsic and extrinsic factors. The HSF1-mediated stress response, in turn, remains persistently mobilized inside cancer cells and is wholly integrated into their malignant state, thereby containing proteotoxic stress and managing to preserve delicate proteostasis. However, this fragile homeostatic state, owing to chronic intrinsic stress, is particularly vulnerable to perturbations. Consequently, protein destabilization, aggregation, and, ultimately, amyloidogenesis, ensue. Biologically, this proteomic chaos is tumor-suppressive, a phenomenon that could be harnessed to conquer malignancy.

In spite of these exciting new developments, many key questions remain outstanding (Box 3). Whereas phosphorylation plays a crucial role in modulating HSF1 activation, recent studies have implicated other posttranslational modifications, such as acetylation and sumoylation, in this process [97,98]. It would be interesting to know whether these modifications contribute to HSF1 activation in cancer cells and whether targeting these modifications could also suppress HSF1. Furthermore, to date all of the functions of HSF1 have been exclusively ascribed to its widely recognized transcriptional mechanism; nevertheless, it remains unknown whether HSF1 could, in fact, act independently of gene regulation. Albeit readily induced in cancer cells, the precise identity of these cancer-associated amyloids remains mysterious. The answer to this question is of great importance for a full comprehension of the amyloidogenic phenomenon of cancer. Moreover, in contrast

to the apparent cell-autonomous effects of HSF1 on oncogenesis, its non-cell-autonomous effects remain poorly understood. An interesting recent study reports that stromal HSF1 activation promotes malignancy through secretion of transforming growth factor β (TGF β) and stromal-derived factor 1 (SDF1) [99]. Nonetheless, further investigations are needed to fully delineate how HSF1 influences malignancy through the tumor microenvironment.

OUTSTANDING QUESTIONS

- Do acetylation and sumoylation impact constitutive activation of HSF1 within cancer cells? If yes, can we target these posttranslational modifications to suppress HSF1 in cancer?
- Does HSF1 have transcription-independent functions? If yes, are these non-canonical modes of action critical to oncogenesis?
- What are the identities of cancer-associated amyloidogenic proteins? How do their normal functions influence oncogenesis?
- Does HSF1 co-opt tumor microenvironments to support malignancy?

Whereas genomic instability is widely recognized as a hallmark of cancer, proteomic instability of cancer has drawn little attention. Now evidence is just beginning to shed light on this emerging horizon in mechanisms of cancer, elucidation of which will not only greatly expand our knowledge of cancer biology but will also unlock new avenues to designing novel anti-neoplastic therapies.

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Glossary

Amyloidogenesis

the process of forming amyloids. Amyloids are protein aggregates that are enriched for highly ordered β -sheet structures and are frequently associated with human neurodegenerative diseases.

Biological stress

a state of cells, tissues, or organisms under disrupted homeostasis of a particular biological process or system. There are a wide variety of stresses, including genotoxic, proteotoxic, oxidative, and metabolic stress, herein defined by the primarily affected biological process or system, such as genome, proteome, oxidants/antioxidants, or metabolism. Of note, many biological stresses are interconnected and one type of stress often triggers other types of stress secondarily. For example, oxidative stress can subsequently cause DNA and

	protein damage, thereby further provoking genotoxic and proteotoxic stress.
Chromatin Immunoprecipitation Sequencing (ChIP-seq)	a technique that combines conventional chromatin immunoprecipitation with massively parallel sequencing. ChIP-seq enables genome-wide mapping of interactions between protein and DNA.
Genetically engineered mouse model	mice with modified genomes through various genetic engineering techniques, including transgenesis, gene knockout, and gene knockin. These mice are often created to model human diseases <i>in vivo</i> .
Malignant transformation	the process during which a normal or pre-cancerous cell undergoes drastic biological changes to become a cancerous cell.
Proteome	the complete collection of proteins expressed by a cell, tissue, or organism.
Proteostasis	First, new polypeptides are produced by ribosomes; subsequently, these nascent polypeptides fold into appropriate three-dimensional conformations with the assistance of heat-shock proteins; and lastly, misfolded or aggregated proteins and proteins reaching the end of their normal lifespan are recognized as wastes and promptly removed from cells via the ubiquitin-proteasomal pathway or the autophagy-lysosomal pathway.
Xenograft mouse model	immunocompromised mice that carry transplanted cells or tissues derived from another species, such as human.

References

1. Gaillard H, et al. Replication stress and cancer. *Nature reviews. Cancer*. 2015; 15:276–289. [PubMed: 25907220]
2. Negrini S, et al. Genomic instability—an evolving hallmark of cancer. *Nature reviews. Molecular cell biology*. 2010; 11:220–228. [PubMed: 20177397]
3. Sosa V, et al. Oxidative stress and cancer: an overview. *Ageing research reviews*. 2013; 12:376–390. [PubMed: 23123177]
4. Gupta-Elera G, et al. The role of oxidative stress in prostate cancer. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation*. 2012; 21:155–162.
5. Reid MA, Kong M. Dealing with hunger: Metabolic stress responses in tumors. *Journal of carcinogenesis*. 2013; 12:17. [PubMed: 24227992]
6. Giuliani CM, Dass CR. Metabolic stress and cancer: is autophagy the common denominator and a feasible target? *The Journal of pharmacy and pharmacology*. 2014; 66:597–614. [PubMed: 24341996]
7. Lim J, Yue Z. Neuronal aggregates: formation, clearance, and spreading. *Developmental cell*. 2015; 32:491–501. [PubMed: 25710535]

8. Labbadia J, Morimoto RI. The Biology of Proteostasis in Aging and Disease. *Annu Rev Biochem.* 2015; 84:435–464. [PubMed: 25784053]
9. Morimoto RI. The heat shock response: systems biology of proteotoxic stress in aging and disease. *Cold Spring Harb Symp Quant Biol.* 2011; 76:91–99. [PubMed: 22371371]
10. Anckar J, Sistonen L. Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu Rev Biochem.* 2011; 80:1089–1115. [PubMed: 21417720]
11. Brandvold KR, Morimoto RI. The chemical biology of molecular chaperones - Implications for modulation of proteostasis. *J Mol Biol.* 2015 pii: S0022-2836(15)00294-6. 10.1016/j.jmb.2015.05.010
12. Smith HL, et al. Molecular chaperones and neuronal proteostasis. *Semin Cell Dev Biol.* 2015; 40:142–152. [PubMed: 25770416]
13. Doyle SM, et al. Protein rescue from aggregates by powerful molecular chaperone machines. *Nature reviews. Molecular cell biology.* 2013; 14:617–629. [PubMed: 24061228]
14. Fujimoto M, Nakai A. The heat shock factor family and adaptation to proteotoxic stress. *The FEBS journal.* 2010; 277:4112–4125. [PubMed: 20945528]
15. Akerfelt M, et al. Heat shock factors: integrators of cell stress, development and lifespan. *Nature reviews. Molecular cell biology.* 2010; 11:545–555. [PubMed: 20628411]
16. Vihervaara A, Sistonen L. HSF1 at a glance. *J Cell Sci.* 2014; 127:261–266. [PubMed: 24421309]
17. Xiao X, et al. HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. *The EMBO journal.* 1999; 18:5943–5952. [PubMed: 10545106]
18. Zhang Y, et al. Targeted disruption of hsf1 leads to lack of thermotolerance and defines tissue-specific regulation for stress-inducible Hsp molecular chaperones. *J Cell Biochem.* 2002; 86:376–393. [PubMed: 12112007]
19. Izu H, et al. Heat shock transcription factor 1 is involved in quality-control mechanisms in male germ cells. *Biol Reprod.* 2004; 70:18–24. [PubMed: 12954729]
20. Raychaudhuri S, et al. Interplay of acetyltransferase EP300 and the proteasome system in regulating heat shock transcription factor 1. *Cell.* 2014; 156:975–985. [PubMed: 24581496]
21. Dai C, et al. Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell.* 2007; 130:1005–1018. [PubMed: 17889646]
22. Dai C, et al. Loss of tumor suppressor NF1 activates HSF1 to promote carcinogenesis. *The Journal of clinical investigation.* 2012; 122:3742–3754. [PubMed: 22945628]
23. Mendillo ML, et al. HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell.* 2012; 150:549–562. [PubMed: 22863008]
24. Ciocca DR, et al. Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. *Arch Toxicol.* 2013; 87:19–48. [PubMed: 22885793]
25. Jego G, et al. Targeting heat shock proteins in cancer. *Cancer Lett.* 2013; 332:275–285. [PubMed: 21078542]
26. Amend SR, Pienta KJ. Ecology meets cancer biology: the cancer swamp promotes the lethal cancer phenotype. *Oncotarget.* 2015; 6:9669–9678. [PubMed: 25895024]
27. Parks SK, et al. Disrupting proton dynamics and energy metabolism for cancer therapy. *Nature reviews. Cancer.* 2013; 13:611–623. [PubMed: 23969692]
28. Zoncu R, et al. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nature reviews. Molecular cell biology.* 2011; 12:21–35. [PubMed: 21157483]
29. Wander SA, et al. Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy. *The Journal of clinical investigation.* 2011; 121:1231–1241. [PubMed: 21490404]
30. Cargnello M, et al. The expanding role of mTOR in cancer cell growth and proliferation. *Mutagenesis.* 2015; 30:169–176. [PubMed: 25688110]
31. Buszczak M, et al. Cellular differences in protein synthesis regulate tissue homeostasis. *Cell.* 2014; 159:242–251. [PubMed: 25303523]
32. Oromendia AB, et al. Aneuploidy causes proteotoxic stress in yeast. *Genes Dev.* 2012; 26:2696–2708. [PubMed: 23222101]

33. Donnelly N, Storchova Z. Causes and consequences of protein folding stress in aneuploid cells. *Cell cycle*. 2015; 14:495–501. [PubMed: 25602365]
34. Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nature reviews. Cancer*. 2014; 14:709–721. [PubMed: 25342630]
35. Jung T, et al. The proteasome and the degradation of oxidized proteins: Part II - protein oxidation and proteasomal degradation. *Redox biology*. 2013; 2C:99–104. [PubMed: 25460724]
36. Reva B, et al. Predicting the functional impact of protein mutations: application to cancer genomics. *Nucleic Acids Res*. 2011; 39:e118. [PubMed: 21727090]
37. Holmberg CI, et al. Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. *The EMBO journal*. 2001; 20:3800–3810. [PubMed: 11447121]
38. Zhang Y, et al. Protein kinase A regulates molecular chaperone transcription and protein aggregation. *PloS one*. 2011; 6:e28950. [PubMed: 22216146]
39. Soncin F, et al. Transcriptional activity and DNA binding of heat shock factor-1 involve phosphorylation on threonine 142 by CK2. *Biochem Biophys Res Commun*. 2003; 303:700–706. [PubMed: 12659875]
40. Kim SA, et al. Polo-like kinase 1 phosphorylates heat shock transcription factor 1 and mediates its nuclear translocation during heat stress. *The Journal of biological chemistry*. 2005; 280:12653–12657. [PubMed: 15661742]
41. Guettouche T, et al. Analysis of phosphorylation of human heat shock factor 1 in cells experiencing a stress. *BMC biochemistry*. 2005; 6:4. [PubMed: 15760475]
42. Chou SD, et al. mTOR is essential for the proteotoxic stress response, HSF1 activation and heat shock protein synthesis. *PloS one*. 2012; 7:e39679. [PubMed: 22768106]
43. Tang Z, et al. MEK guards proteome stability and inhibits tumor-suppressive amyloidogenesis via HSF1. *Cell*. 2015; 160:729–744. [PubMed: 25679764]
44. Wang X, et al. Phosphorylation of HSF1 by MAPK-activated protein kinase 2 on serine 121, inhibits transcriptional activity and promotes HSP90 binding. *The Journal of biological chemistry*. 2006; 281:782–791. [PubMed: 16278218]
45. Wang X, et al. 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*. 2003; 23:6013–6026. [PubMed: 12917326]
46. Chu B, et al. Transcriptional activity of heat shock factor 1 at 37 degrees C is repressed through phosphorylation on two distinct serine residues by glycogen synthase kinase 3 and protein kinases Calpha and Czeta. *The Journal of biological chemistry*. 1998; 273:18640–18646. [PubMed: 9660838]
47. Samatar AA, Poulikakos PI. Targeting RAS-ERK signalling in cancer: promises and challenges. *Nature reviews. Drug discovery*. 2014; 13:928–942. [PubMed: 25435214]
48. Calvo F, et al. The Ras-ERK pathway: understanding site-specific signaling provides hope of new anti-tumor therapies. *BioEssays : news and reviews in molecular, cellular and developmental biology*. 2010; 32:412–421.
49. Rauen KA. The RASopathies. *Annual review of genomics and human genetics*. 2013; 14:355–369.
50. Fernandez-Medarde A, Santos E. Ras in cancer and developmental diseases. *Genes & cancer*. 2011; 2:344–358. [PubMed: 21779504]
51. Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. *The Lancet. Neurology*. 2014; 13:834–843. [PubMed: 25030515]
52. Gripp KW, Lin AE. Costello syndrome: a Ras/mitogen activated protein kinase pathway syndrome (rasopathy) resulting from HRAS germline mutations. *Genetics in medicine : official journal of the American College of Medical Genetics*. 2012; 14:285–292. [PubMed: 22261753]
53. Roberts AE, et al. Noonan syndrome. *Lancet*. 2013; 381:333–342. [PubMed: 23312968]
54. Sarkozy A, et al. Leopard syndrome. *Orphanet journal of rare diseases*. 2008; 3:13. [PubMed: 18505544]
55. Dai S, et al. Suppression of the HSF1-mediated proteotoxic stress response by the metabolic stress sensor AMPK. *The EMBO journal*. 2015; 34:275–293. [PubMed: 25425574]

56. Hardie DG. AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Curr Opin Cell Biol.* 2015; 33:1–7. [PubMed: 25259783]
57. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nature cell biology.* 2011; 13:1016–1023. [PubMed: 21892142]
58. Hardie DG. The LKB1-AMPK pathway-friend or foe in cancer? *Cancer cell.* 2013; 23:131–132. [PubMed: 23410967]
59. Korsse SE, et al. Targeting LKB1 signaling in cancer. *Biochim Biophys Acta.* 2013; 1835:194–210. [PubMed: 23287572]
60. Gwinn DM, et al. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell.* 2008; 30:214–226. [PubMed: 18439900]
61. Min JN, et al. Selective suppression of lymphomas by functional loss of Hsf1 in a p53-deficient mouse model for spontaneous tumors. *Oncogene.* 2007; 26:5086–5097. [PubMed: 17310987]
62. Jin X, et al. Heat shock transcription factor 1 is a key determinant of HCC development by regulating hepatic steatosis and metabolic syndrome. *Cell metabolism.* 2011; 14:91–103. [PubMed: 21723507]
63. Gabai VL, et al. 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.* 2012; 32:929–940. [PubMed: 22215620]
64. Xi C, et al. Heat shock factor Hsf1 cooperates with ErbB2 (Her2/Neu) protein to promote mammary tumorigenesis and metastasis. *The Journal of biological chemistry.* 2012; 287:35646–35657. [PubMed: 22847003]
65. Meng L, et al. Heat-shock transcription factor HSF1 has a critical role in human epidermal growth factor receptor-2-induced cellular transformation and tumorigenesis. *Oncogene.* 2010; 29:5204–5213. [PubMed: 20622894]
66. Fang F, et al. Heat shock factor 1 promotes invasion and metastasis of hepatocellular carcinoma in vitro and in vivo. *Cancer.* 2012; 118:1782–1794. [PubMed: 22009757]
67. Chen Y, et al. Targeting HSF1 sensitizes cancer cells to HSP90 inhibition. *Oncotarget.* 2013; 4:816–829. [PubMed: 23615731]
68. Fujimoto M, et al. RPA assists HSF1 access to nucleosomal DNA by recruiting histone chaperone FACT. *Mol Cell.* 2012; 48:182–194. [PubMed: 22940245]
69. Nakamura Y, et al. Heat shock factor 1 is required for migration and invasion of human melanoma in vitro and in vivo. *Cancer Lett.* 2014; 354:329–335. [PubMed: 25194503]
70. Scott KL, et al. Proinvasion metastasis drivers in early-stage melanoma are oncogenes. *Cancer cell.* 2011; 20:92–103. [PubMed: 21741599]
71. Toma-Jonik A, et al. Active heat shock transcription factor 1 supports migration of the melanoma cells via vinculin down-regulation. *Cell Signal.* 2015; 27:394–401. [PubMed: 25435429]
72. Heimberger T, et al. The heat shock transcription factor 1 as a potential new therapeutic target in multiple myeloma. *Br J Haematol.* 2013; 160:465–476. [PubMed: 23252346]
73. Dudeja V, et al. Prosurvival role of heat shock factor 1 in the pathogenesis of pancreatobiliary tumors. *American journal of physiology. Gastrointestinal and liver physiology.* 2011; 300:G948–955. [PubMed: 21330448]
74. Kim SA, et al. The antitumor effect of PLK1 and HSF1 double knockdown on human oral carcinoma cells. *Int J Oncol.* 2010; 36:867–872. [PubMed: 20198330]
75. Luo J, et al. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell.* 2009; 136:823–837. [PubMed: 19269363]
76. Dai C, et al. Proteotoxic stress of cancer: implication of the heat-shock response in oncogenesis. *J Cell Physiol.* 2012; 227:2982–2987. [PubMed: 22105155]
77. Miyajima N, et al. The HSP90 inhibitor ganetespib synergizes with the MET kinase inhibitor crizotinib in both crizotinib-sensitive and -resistant MET-driven tumor models. *Cancer Res.* 2013; 73:7022–7033. [PubMed: 24121490]
78. Workman P, van Montfort R. EML4-ALK fusions: propelling cancer but creating exploitable chaperone dependence. *Cancer discovery.* 2014; 4:642–645. [PubMed: 24891363]

79. Li D, et al. A gain-of-function mutant p53-HSF1 feed forward circuit governs adaptation of cancer cells to proteotoxic stress. *Cell death & disease*. 2014; 5:e1194. [PubMed: 24763051]
80. Trinklein ND, et al. The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. *Mol Biol Cell*. 2004; 15:1254–1261. [PubMed: 14668476]
81. Kolch W. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol*. 2005; 6:827–837. [PubMed: 16227978]
82. Qian SB, et al. mTORC1 links protein quality and quantity control by sensing chaperone availability. *J Biol Chem*. 2010; 285:27385–27395. [PubMed: 20605781]
83. Hamanaka RB, Chandel NS. Targeting glucose metabolism for cancer therapy. *J Exp Med*. 2012; 209:211–215. [PubMed: 22330683]
84. Santagata S, et al. Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. *Science*. 2013; 341:1238303. [PubMed: 23869022]
85. Wu N, et al. AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. *Mol Cell*. 2013; 49:1167–1175. [PubMed: 23453806]
86. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer*. 2007; 7:763–777. [PubMed: 17882277]
87. O'Callaghan-Sunol C, Sherman MY. Heat shock transcription factor (HSF1) plays a critical role in cell migration via maintaining MAP kinase signaling. *Cell Cycle*. 2006; 5:1431–1437. [PubMed: 16855393]
88. Nagai N, et al. Quercetin suppresses heat shock response by down regulation of HSF1. *Biochem Biophys Res Commun*. 1995; 208:1099–1105. [PubMed: 7702609]
89. Ohnishi K, et al. Effects of a heat shock protein inhibitor KNK437 on heat sensitivity and heat tolerance in human squamous cell carcinoma cell lines differing in p53 status. *Int J Radiat Biol*. 2004; 80:607–614. [PubMed: 15370972]
90. Westerheide SD, et al. Triptolide, an inhibitor of the human heat shock response that enhances stress-induced cell death. *The Journal of biological chemistry*. 2006; 281:9616–9622. [PubMed: 16469748]
91. Yoon YJ, et al. KRIBB11 inhibits HSP70 synthesis through inhibition of heat shock factor 1 function by impairing the recruitment of positive transcription elongation factor b to the hsp70 promoter. *The Journal of biological chemistry*. 2011; 286:1737–1747. [PubMed: 21078672]
92. Santagata S, et al. Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. *Science*. 2013; 341:1238303. [PubMed: 23869022]
93. Salamanca HH, et al. Inhibiting heat shock factor 1 in human cancer cells with a potent RNA aptamer. *PloS one*. 2014; 9:e96330. [PubMed: 24800749]
94. Santagata S, et al. High levels of nuclear heat-shock factor 1 (HSF1) are associated with poor prognosis in breast cancer. *Proc Natl Acad Sci U S A*. 2011; 108:18378–18383. [PubMed: 22042860]
95. Ishiwata J, et al. State of heat shock factor 1 expression as a putative diagnostic marker for oral squamous cell carcinoma. *Int J Oncol*. 2012; 40:47–52. [PubMed: 21879256]
96. Engerud H, et al. High level of HSF1 associates with aggressive endometrial carcinoma and suggests potential for HSP90 inhibitors. *Br J Cancer*. 2014; 111:78–84. [PubMed: 24853175]
97. Westerheide SD, et al. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science*. 2009; 323:1063–1066. [PubMed: 19229036]
98. Hong Y, et al. Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. *The Journal of biological chemistry*. 2001; 276:40263–40267. [PubMed: 11514557]
99. Scherz-Shouval R, et al. The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. *Cell*. 2014; 158:564–578. [PubMed: 25083868]
100. McMillan DR, et al. Heat shock transcription factor 2 is not essential for embryonic development, fertility, or adult cognitive and psychomotor function in mice. *Mol Cell Biol*. 2002; 22:8005–8014. [PubMed: 12391166]
101. Fujimoto M, et al. A novel mouse HSF3 has the potential to activate nonclassical heat-shock genes during heat shock. *Mol Biol Cell*. 2010; 21:106–116. [PubMed: 19864465]

102. Fujimoto M, et al. HSF4 is required for normal cell growth and differentiation during mouse lens development. *The EMBO journal*. 2004; 23:4297–4306. [PubMed: 15483628]
103. Ostling P, et al. Heat shock factor 2 (HSF2) contributes to inducible expression of hsp genes through interplay with HSF1. *The Journal of biological chemistry*. 2007; 282:7077–7086. [PubMed: 17213196]
104. Kallio M, et al. Brain abnormalities, defective meiotic chromosome synapsis and female subfertility in HSF2 null mice. *The EMBO journal*. 2002; 21:2591–2601. [PubMed: 12032072]

TRENDS

- The heat shock factor 1 (HSF1)-mediated proteotoxic stress response (PSR) is an evolutionarily conserved powerful transcriptional program that guards the cellular proteome against the dangers of misfolding and aggregation.
- Cancerous cells suffer chronic proteotoxic stress from without and within.
- The HSF1-mediated PSR is constitutively mobilized within cancerous cells.
- HSF1 plays a pivotal role in preserving proteomic stability of cancer, thereby enabling robust malignant transformation.
- Disrupting fragile proteostasis in cancer provokes proteomic chaos and tumor-suppressive amyloidogenesis, representing a novel anti-neoplastic therapeutic strategy.

Figure 1. Multi-step activation of HSF1 by stress

Under non-stressed conditions, inactive monomeric HSF1 remains repressed by the products of its own transcriptional targets, HSPs, in the cytoplasm. Proteotoxic stressors, such as heat shock, trigger dissociation of the repressive protein complex and release of monomeric HSF1. Subsequently, HSF1 undergoes trimerization, nuclear translocation, and posttranslational modifications including phosphorylation, acetylation, and sumoylation. Among these modifications, phosphorylation is well documented and has been shown to critically regulate HSF1 activation. In the nucleus, activated trimeric HSF1, with the assistance of the single-stranded DNA-binding protein RPA, the chromatin-remodeling enzyme BRG1, and the histone chaperone FACT, accesses and binds to HSEs, which subsequently trigger recruitment of a pre-initiation complex that comprises RNA polymerase II (RNA Pol II) and general transcription factors (TFII). Abbreviations:

HDAC6, histone deacetylase 6; RPA, replication protein A; BRG1, brahma related gene 1; FACT, facilitates chromatin transcription. E1, 2, n: *HSP* gene exons.

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Figure 2. Oncogenic and tumor-suppressive signaling intimately regulates HSF1

(A) In healthy cells, mitogenic RAS signaling activates HSF1 through MEK-mediated Ser326 phosphorylation. ERK, the canonical substrate for MEK, suppresses MEK-mediated HSF1 activation through inhibitory Thr292/386 phosphorylation of MEK, in a negative feedback manner. Congruent with its role as a negative regulator of RAS, the tumor suppressor NF1 inactivates HSF1. In addition, tumor-suppressive LKB1 signaling could inactivate HSF1 through AMPK-mediated Ser121 phosphorylation. AMPK, a pivotal sensor of energy depletion, critically regulates the MSR. Through mobilization of AMPK, metabolic stressors, including metformin and nutrient deprivation, inactivate HSF1. Abbreviations: S, serine; T, threonine; Y, tyrosine. (B) Germline mutations in the *NF1* and *LKB1* gene cause Neurofibromatosis type I and Peutz-Jeghers Syndrome, respectively, in humans. Afflicted humans are predisposed to cancer. While in *NF1*-deficient cells hyper-activated oncogenic RAS signaling causes constitutive Ser326 phosphorylation and activation of HSF1, in *LKB1*-deficient cells hypo-activation of AMPK leads to impaired HSF1 Ser121 phosphorylation, a modification inhibitory to HSF1 activation.

Figure 3. HSF1 guards cancer proteomic stability and enables effective stress adaptation

(A) Cancer cells with constitutive HSF1 activation possess abundant chaperoning capacity, thereby averting proteomic instability. Moreover, HSF1 regulates numerous non-*HSP* genes that are engaged in diverse cellular processes, thereby orchestrating a preemptive systemic response that empowers cells to promptly adapt to stress. (B) Impairment of HSF1 activation depletes cellular chaperoning capacity, inevitably provoking protein destabilization and misfolding. Accumulated misfolded proteins further form either amorphous aggregates or, ultimately, amyloids. In addition, the stress-adapting ability of cancer cells is severely compromised. As a consequence, robust malignant phenotypes can no longer be sustained. m⁷G: mRNA 7-methylguanosine cap; AAAAA: mRNA poly(A) tail; HSP: heat-shock protein; HSF1: heat shock factor 1; Ub: ubiquitin.