Expert Opinion

Inhibiting the transcription factor HSF1 as an anticancer strategy

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Background: In mammals, the cytoprotective heat-shock response is regulated primarily by heat shock factor 1 (HSF1). Unfortunately, the effects of HSF1 also support the ability of cancer cells to accommodate imbalances in signaling and alterations in DNA, protein and energy metabolism associated with oncogenesis. The malignant lifestyle confers dependence on this 'non-oncogene', suggesting a therapeutic role for HSF1 inhibitors. *Objective/methods*: We begin with an overview of how HSF1 affects cancer biology and how its activity is regulated. We then summarize progress in discovery and development of HSF1 inhibitors, their current limitations and potential as anticancer agents with a fundamentally different scope of action from other clinically validated modulators of protein homeostasis. *Results/conclusions*: It is likely that within the next 5 years usable inhibitors of HSF1 will be identified and in early pre-clinical evaluation.

Keywords: cancer, chaperone, heat shock factor, non-oncogene, stress response

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1. Introduction

The recent entry of numerous inhibitors of heat shock protein 90 (HSP90) into cancer clinical trials serves as a welcome milestone on the long road to understanding the role of heat-shock proteins (HSP) in cancer and how they might be targeted for therapeutic benefit [1,2]. A similar journey is just beginning for heat shock factor 1 (HSF1), the master transcription factor regulating inducible heat-shock gene expression. Important areas of overlap do exist, but the effect of HSF1 on oncogenesis extends far beyond its ability to increase the expression of HSP90 and the other major HSP classes. Indeed, HSF1 coordinates an ancient, genome wide-transcriptional program known as the heat-shock response that not only restores the normal protein folding environment, but alters signaling pathways and modulates metabolism to enhance cell survival under stress. Such stresses can be imposed by hostile environmental conditions including hyperthermia, ischemia in poorly perfused organs such as the heart or brain, or acidotic, nutrient-deprived conditions within a tumor mass. Less widely appreciated, they can be imposed by internal, cell-autonomous processes such as the accumulation of misfolded proteins during normal aging, the overexpression of mutant, misfolding-prone oncoproteins or the drastic alterations in DNA, protein and energy metabolism that they drive. Since many of these stress conditions are relevant to the pathophysiology of common human diseases ranging from cancer to neurodegeneration, pharmacological modulation of HSF1 function, both positively and negatively, has numerous therapeutic implications that have yet to be exploited [3].

2. Regulation of HSF1 and the heat-shock response in cells

Regulation of the heat shock response is complex, with multiple layers of redundancy and feedback control in effect at the molecular level (reviewed in [4]). Clearly

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many questions remain, but our emerging understanding of how HSF1 activity is regulated at the molecular level suggests multiple points of possible intervention by drug-like compounds and supports the feasibility of identifying selective inhibitors of its function. In humans, three heat shock factors (HSF1, HSF2 and HSF4) have been identified that play a role in transcriptional control of heat shock protein expression. While sharing only 40% sequence identity, all HSFs bind to consensus heat shock elements (HSE) within the promoter regions of the known HSP genes upstream of their transcriptional start sites. In species from yeast to man, classical HSEs consist of multiple adjacent inverted arrays of the binding site (5′-nGAAn-3′), but non-canonical sites have also been described. In *Drosophila* cells where the polytene chromosomes make possible direct microscopic observation of HSF interaction with DNA in live cells, the protein is localized to the nucleus under basal conditions, but it translocates from nucleoplasm to specific chromosomal loci after heat shock where it remains stably bound and recruits RNA polymerase II (Pol II) [5]. To efficiently transcribe genes such as HSP70 after heat shock, Pol II must overcome barriers imposed by nucleosomes and higher-order chromatin structure. Inhibitory RNA (RNAi) depletion experiments, again in *Drosophila* cells, indicate that HSF plays an essential role in this re-modeling process, independent of Pol II transcriptional activity [6]. Although it has been underappreciated and little studied, HSF1 can act as a transcriptional repressor as well as an activator depending on the spacing of HSEs to which it binds and the cohort of additional factors which it recruits [7,8]. Furthermore, in addition to direct regulation of gene targets, HSF1 also indirectly affects gene expression by regulating the expression of other transcription factors and *trans*-acting proteins [9,10].

Although HSF2 and HSF4 play some role as modulators, HSF1 is clearly the dominant factor controlling cellular responses to a diverse array of stresses. These include heat and other proteotoxic stressors such as heavy metals and oxidative agents (reviewed in [11]). Genetic knockout of *Hsf1* completely abrogates induction of the stress response in transgenic mice following heat shock. Importantly, normal basal expression of the major HSP classes is preserved (**Figure 1**, left panel). This makes HSF1 dispensable for growth and survival under controlled laboratory conditions [12]. *Hsf1*-knockout embryos suffer from defects in development of the chorioallantoic placenta that support their gestation and are recovered from crosses in lower numbers than expected. Upon sexual maturation, female, but not male, knockout mice are infertile due to defects in germ cell development [13,14]. Other than a smaller overall body size throughout life, however, knockout mice display no other gross organ system abnormalities. Indeed, in the absence of acute stressors such as high temperature or endotoxin challenge, they live until advanced old age. Whether they modestly pre-decease or outlive wild-type littermates varies somewhat with strain background [15]. It is of note that these findings in mice stand in sharp contrast to the simpler model organism *Caenorhabditis elegans* where genetic compromise of the relevant HSF1 ortholog significantly shortens lifespan [16,17]. The reason for the disparity is unknown, but further work addressing the issue could provide valuable insights into unique aspects of HSF1 function in mammals.

Because of its central role in regulating the heat-shock response, much effort has been directed at understanding HSF1 activation at the molecular level. A cartoon highlighting the key elements of our current understanding of how HSF1 function is regulated in vertebrates is provided in **Figure 1** (right panel), but the picture is far from complete. Contrary to popular conception, HSF1 is not always predominantly localized to the cytosol under basal conditions. Rather, its basal sub-cellular distribution can vary amongst cell types and between different tissues [18]. As a result, nuclear localization by immunostaining as an indicator of activation status must be interpreted cautiously with histological context in mind. In addition to localization, the phosphorylation status of specific epitopes in HSF1 may prove a useful adjunct in assessing activation status. A systematic study of HSF1 phosphorylation sites using a combination of mass spectrometry, alanine scanning and reporter assays identified 12 serine residues, but no threonine or tyrosine residues that are inducibly phosphorylated following heat shock [19]. Amongst these residues, phosphorylation of Ser326 seems to play the most important functional role and, given the availability of phospho-specific antibodies to this epitope, it might provide the most useful indicator of activation status.

Immunostaining and biochemical fractionation experiments in mammalian cells have shown that non-activated HSF1 shuttles continuously between the cytoplasm and nucleus with its apparent localization and turnover rate dependent on the relative dwell time spent in these two compartments [20]. This biology is reminiscent of another primordial transcription factor, the estrogen receptor which resides predominantly in the nucleus in the absence of activating signals (estrogens). While in the non-activated state, HSF1 undergoes iterative interactions with an HSP90-based chaperone complex that includes a protein deacetylase, histone deacetylase 6 (HDAC6) [21]. Recent work indicates that HDAC6 somehow senses the accumulation of misfolded, ubiquitinated proteins in cells and participates in their sequestration into aggresomes. This induces expression of the major cellular chaperones in a manner independent of HDAC6 deacetylase activity, but involves the dissociation of a repressive HDAC6–HSF1–HSP90 complex and subsequent HSF1 activation (**Figure 1**). It is of note that HSP90 inhibitors break the normal cycling of this regulatory machinery and thereby provide one mechanism to induce the heat shock response, which is being explored for its therapeutic potential in protein-aggregation-associated neurodegenerative disorders [22-24].

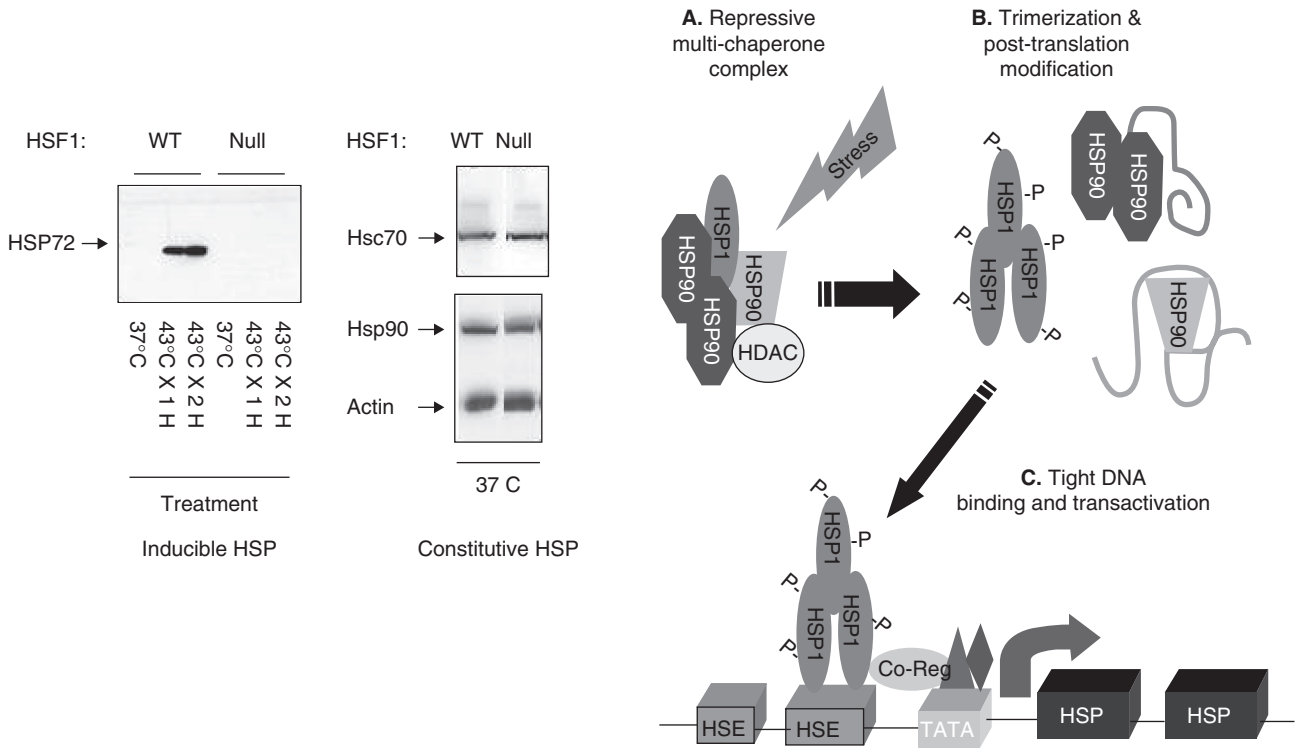

Figure 1. Heat shock factor 1 (HSF1)-mediated regulation of gene expression. Left panel: HSF1 is required for induction of the heat-shock response, but not for maintenance of basal HSP levels. Cell lysates were prepared from wild type mouse embryonic fibroblasts (WT) or fibroblasts derived from homozygous *Hsf1*-knockout littermates (Null). Cells were cultured at standard temperature (37 ˚C) or heat-shocked at 43 ˚C for the times indicated and then returned to standard temperature overnight prior to lysis. Equal amounts of total cellular protein were blotted for the highly inducible heat shock protein 70 (HSP70) isoform (HSP72). In addition, relative cellular levels of the constitutively expressed HSP70 isoform HSC70 and HSP90 were evaluated in both genotypes under basal conditions. Actin served as a loading control. Right panel: Cartoon summarizing key features of the most widely accepted model for regulation of HSF1 transcriptional activating activity. **A**. While in the non-activated state, HSF1 undergoes iterative interactions with a repressive HSP90-based chaperone complex that includes the protein deacetylase histone deacetylase 6 (HDAC6). **B**. Proteotoxic stress such as heat leads to the accumulation of non-native/denatured proteins (unbroken lines). Chaperones including but not limited to HSP70 and HSP90 are recruited to damaged proteins, where they prevent aggregation and assist in renaturation. If damage is unrecoverable, they may also assist in presentation to the ubiquitin-proteasome degradation pathway (not depicted). The recruitment of chaperones to assist in these functions titers them away from their metastable association with HSF1. This allows HSF1 to accumulate as a trimer and undergo extensive post-translational modification, most notably phosphorylation on multiple serine residues. **C**. Phosphorylated HSF1 trimers bind tightly to consensus heat shock elements (HSE) within the promoter regions of target genes. Binding results in recruitment of additional co-regulators (Co-Reg) as well as elements of the basal transcriptional machinery (represented by a diamond and a triangle). In the case of inducible HSP, the result is transcriptional activation and a rapid rise in their cellular levels. After protein homeostasis is restored with their assistance, these HSP return to acting as repressors of HSF1 and the response is terminated (not depicted).

In response to proteotoxic stressors such as heat, chaperones are titered away from HSF1 to assist damaged, non-native proteins. HSF1 becomes freed from repressive chaperone interactions to form homotrimers and to undergo extensive phosphorylation [25]. Both events are required for HSF1 to bind promoter regions tightly and regulate gene expression after heat shock. Although heat shock is the best studied, alternative modes of activating HSF1 can have profound effects that vary with the responsible stimulus. For example, it is becoming evident that the activation of HSF1 during malignant transformation as compared with heat shock is associated with overlapping but distinct patterns of post-translational modification to the protein. Work is only beginning, but differences in the recruitment of co-regulators and the complement of genes whose expression is subsequently altered are also likely. Indeed, the specific co-activators and co-repressors that are recruited in the process of HSF1 activation are largely unknown, although death domain-associated protein (DAXX) has been implicated on the basis of yeast two-hybrid and human cell line data [26]. Although non-essential, HSF2 can also physically interact with HSF1 in heteromeric complexes to help fine tune the transcriptional regulation of HSP genes [27]. Interestingly, HSF2 has been shown to selectively 'bookmark' the inducible HSP70

gene during mitosis, an epigenetic mechanism which allows the gene to re-establish transcriptional competence very early after cell division in the G1 phase of the cell cycle [28].

Multiple regulators have been reported to act directly on HSF1 and modulate its activation state. (summarized in **Table 1**). HSF1 activity is positively regulated by phosphorylation on serine residues by the kinases polo-like kinase 1 (PLK1) [29,30] and calcium/calmodulin-dependent protein kinase II (CaMKII), potentially in a tissue-specific manner [31,32]. The protein phosphatase PP5 physically interacts with HSF1 and negatively regulates its activity [33]. In contrast, phosphorylation on certain HSF1 residues mediated by glycogen synthase kinase 3 beta (GSK-3β) [34], PKC isoforms [35] and extracellular signal-regulated kinase (ERK1) [36,37] repress HSF1 activity, at least in part by recruiting the binding of 14-3-3 proteins and the sequestration of HSF1 in the cytosol [38]. Murine thymoma viral (v-akt) oncogene homolog-1 (AKT)-mediated inhibitory phosphorylation of GSK-3β enhances the activation of HSF1. Such activation, either directly or through the consequent upregulation of HSP levels probably contributes significantly to the anti-apoptotic effects of AKT that are well-recognized to facilitate oncogenesis [39,40].

A plethora of other mechanisms are likely to impinge on HSF1 regulation. The stress-induced covalent addition of small ubiquitin-related modifier (SUMO) to HSF1 by the ubiquitin-conjugating enzyme UBC9 in a phosphorylation-dependent manner has been reported, but the precise role of this modification remains unclear [41-43]. An intriguing non-coding RNA [heat shock related 1(HSR1)] has been reported to positively regulate HSF1 activity and link collapse of the cytoskeleton (a feature of heat shock) with HSF1 activation [44]. Interestingly a synthetic RNA aptamer has been reported that binds to HSF1 in a manner distinct from that in which the transcription factor binds DNA but which inhibits its transactivating activity in whole cell lysate [45]. Although useful for basic investigations, the therapeutic potential of such reagents may be limited. In mammals, the small HSF1-binding protein HSBP1 inhibits HSF1 activity and may be involved in extinguishing the heat shock response after it has been initiated [46]. Acetylation of HSF1 on specific lysine residues also plays a role in attenuating the response [47].

Finally, chaperones also return to acting as repressors of HSF1 after their levels have risen and protein homeostasis is restored. This provides a potential mechanism for autoregulation of the heat-shock response [48-50]. As a case in point, HSP70 has recently been shown to interact with the transcriptional co-repressor repressor element 1-silencing transcription factor co-repressor (CoREST) to form a complex that assists in terminating the response [51].

3. The role of HSF1 in cancer: beyond regulation of HSP expression

Elevated levels of one or more major heat shock protein classes (e.g., HSP90, HSP70, HSP60, HSP40 and HSP27) have been documented in many types of cancers over the years [52]. Prognostic significance of elevation of certain HSP in specific types of cancer has been reported, but the immunohistochemical evaluation of heat shock protein levels is not performed routinely in clinical practice due to lack of power as an independent predictor of outcome. Constitutive activation of HSF1 during tumorigenesis has been invoked to explain these observations, but the mechanisms remain unclear. One possibility is that an increased substrate burden is imposed on the heat shock protein/chaperone machinery by the array of mutated oncoproteins commonly overexpressed in many cancers. Support for this hypothesis comes from the observation that unlike its status in normal cells, the HSP90-based chaperone machinery is pressed to operate at its maximal capacity in cancer cells, a situation that also sensitizes them to HSP90 inhibition by drugs [53].

Mechanisms more specific than generic substrate overload are also likely to be involved in elevating HSP levels. The oncoprotein MYC can activate the promoters of the major HSP [54] and HSF1 can be activated by signaling via oncogenic erbB-family kinases [40]. In addition, HSP expression may normally be repressed by the tumor suppressors p53 or p63 and become derepressed upon the loss of their function, something commonly seen in many cancers [52]. Aneuploidy, a characteristic of most human cancers seems to activate HSF1 by mechanisms that remain to be defined [55,56]. Surprisingly, the hostile tumor microenvironment probably plays a relatively limited role in activating HSF1 and is more relevant to activation of the unfolded protein response (UPR) which up-regulates endoplasmic reticulum-resident chaperones. Prostate cancer cells show lower HSP levels when grown as tumor xenografts than as monolayer cultures [57], supporting cell autonomous factors, rather than the tumor microenvironment, as being primary activators of HSF1.

By whatever mechanisms it may occur, constitutive activation of HSF1 does not fully explain HSP overexpression in cancer cells. Genes for the major HSPs all contain conserved HSF1-binding elements within their typically complex promoter regions, but considerable variation is seen in which HSP classes are increased in different tumor histologies. Even more telling, genetic knockdown of HSF1 fails to reduce HSP levels in many cancer cell lines to the normal basal levels seen in non-transformed cells (Whitesell L & Lindquist S unpublished). From a therapeutic perspective, inhibitors of HSF1, even if highly effective might not reduce HSP over-expression to 'normal' levels and yet might still exert potent anticancer activity by a variety of other mechanisms. As a corollary, HSP reduction in tumors is unlikely to provide the best surrogate endpoint for monitoring the efficacy of HSF1 inhibitors in clinical trials.

If regulating the expression of the major HSPs is not the whole answer, how else might HSF1 be involved in cancer initiation or progression at the molecular level? We recently reported that eliminating HSF1 by genetic

Table 1. Proximal regulators of heat shock factor 1 (HSF1) activation.

Positive regulators	Biochemical activity	Ref.
PLK1	Kinase	[29,30]
CaMKII	Kinase	[31,32]
HSR1	Non-coding RNA	[44]
eEF1A	Translation elongation	[44]
SIRT1	Protein deacetylase	[47]
Negative regulators		
HDAC6	Protein deacetylase	[21]
PP5	Phosphatase	[33]
GSK-3β	Kinase	[34,36]
PKC	Kinase	[35,36]
ERK1	Kinase	[36,37]
UBC9	SUMO-conjugating enzyme	[41-43]
HSBP1	Binding partner	[46]
Heat shock proteins	Molecular chaperones	[48-50]

CaMKII: Calcium/calmodulin-dependent protein kinase II; eEF1A: Eukaryotic translation elongation factor 1 alpha; ERK1: Extracellular-signal-regulated kinase 1; GSK-3β: Glycogen synthase kinase 3 beta; HDAC6: Histone deacetylase 6; HSBP1: HSF1-binding protein 1; HSR1: Heat-shock related 1; PLK1: Polo-like kinase 1; PP5: Protein phosphatase 5; SIRT1: Sirtuin; SUMO: Small ubiquitin-related modifier; UBC9: Ubiquitin-conjugating enzyme 9.

techniques dramatically protects mice from tumor formation induced by clinically relevant examples of the major mechanisms of oncogenesis; activating mutation of a *RAS* oncogene (**Figure 2**) or a loss of tumor suppressor function (hot spot mutation of *p53*). By orchestrating a broad network of core cellular functions that include proliferation, survival, protein synthesis and glucose metabolism, HSF1 greatly enhances the efficiency of oncogenic transformation [58]. Individually, the effects of HSF1 on any one cellular function might have only a modest influence on transformation. But acting in concert with each other, the summation of effects can have a profound impact, making adaptation to the malignant lifestyle possible. Another investigator has reported a more subtle effect of HSF1-deficiency on tumorigenesis, specifically an altered spectrum of tumor types arising in *p53*-homozygous-knockout mice, but not a decline in overall tumor incidence [59]. The discrepancy may well be due to technical differences between the less clinically relevant mouse model used by this group and the models that we have examined. Specifically, homozygous deletion of *p53* is rarely encountered in clinical cancers. Instead, as captured in the model used for our studies, the far more common situation is mutation of one *p53* allele at 'hot spots' within the region encoding its DNA-binding domain and subsequent loss of heterozygosity at the other allele. Indeed, a profound impact of HSF1 on tumorigenesis in general, not just a shift in tumor spectrum is confirmed by our finding that human cancer cell lines of diverse histological origin (breast, prostate, cervix, kidney and nerve sheath) and molecular pathology exhibit a much greater dependence on HSF1 to maintain proliferation and survival as compared with their non-transformed counterparts [58].

Dramatically supporting these functional insights into HSF1's role in oncogenesis, recent genomic studies confirm that HSF1 regulates a broad spectrum of non-HSP genes in organisms ranging from yeast to *Drosophila* and man [60-63]. Genome-wide analysis of human cancer cells (HeLa) using a combination of expression profiling, chromatin immunoprecipitation (ChIP) and RNAi-mediated knockdown has demonstrated HSF1 association under basal conditions with many genes that are not upregulated by physical heat shock [10]. Whether the same holds true in non-transformed cells is a key question yet to be answered. Interestingly, GO (Gene Ontology) classification of the genes bound by HSF1 that did not induce with heat shock showed highly significant enrichment for categories including lipid and cholesterol metabolism, regulation of the cell cycle, and cell proliferation. Although physical heat shock did not increase expression of these genes, RNAi-mediated knockdown of HSF1 significantly decreased the expression of many of them [10].

Thus, rather than simply increasing the expression of HSPs under stressful conditions, HSF1 seems to maintain the expression of many genes that are involved in critical cellular functions and that need to be preserved under stressful conditions if cells are to survive. In this role, HSF1 acts as neither a classical oncogene nor tumor suppressor. Indeed, neither enforced over-expression nor knockout of HSF1 directly drives transformation. Instead, these data indicate that HSF1, as part of its ancient role in enhancing survival is poised to modulate an entire network of cellular functions that enable tumorigenesis. This role renders cancer cells uniquely dependent on HSF1 and provides a striking example of the recently described phenomenon of 'non-oncogene addiction' [64].

4. Targeting HSF1 function to treat cancer

HSF1 provides protection against ischemia/reperfusion injury, endotoxin-induced shock and neurodegenerative disorders. [16,17,65,66]. Based on our findings and those of others, inhibiting HSF1 activation could provide a multifaceted and broadly effective anticancer strategy, but might impair cellular ability to respond to other acute disease processes such as infection or accelerate the progression of neurodegenerative diseases. This trade-off holds true for other centrally poised drug targets such as the proteasome. Inhibition is associated with promising anticancer activity [67], but in some situations it could exacerbate other disease processes [68]. For HSF1 as for the proteasome, this dichotomy should be manageable by limiting the duration and extent

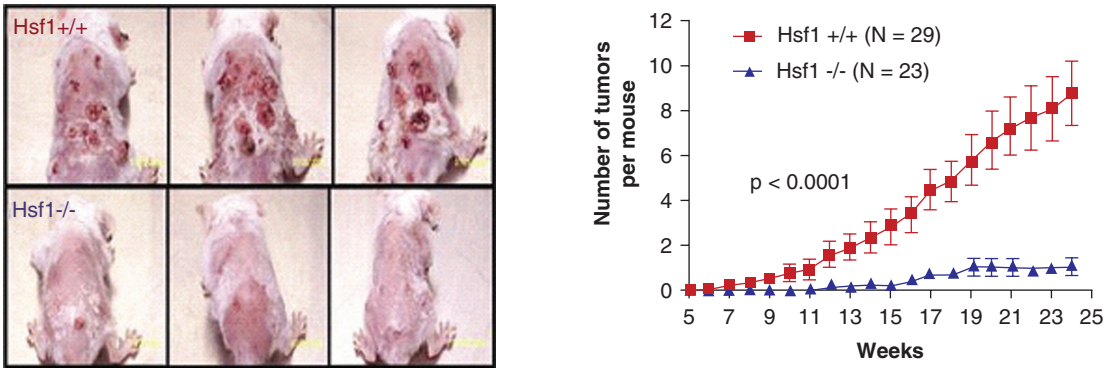

Figure 2. Heat shock factor 1 (HSF1) deficiency suppresses chemical skin carcinogenesis driven by activating mutations of *H-ras*. Left panel: Representative images of skin tumors developing 25 weeks after topical application of the mutagen dimethylbenzanthracene (DMBA) to wild-type ($Hsf1^{+/+}$) and homozygous knockout ($Hsf1^{-/-}$) mice. Right panel: Quantitation of the markedly reduced tumor burden developing in Hsf1-knockout versus Hsf1 wild-type mice after DMBA application.
Figure adapted from [58]. Used by permission of Cell Press/Elsevier.

of inhibitor exposure. For example, administering compounds in pulsed fashion or using agents that do not cross the blood-brain barrier could avoid potential exacerbation of aging-related neurodegenerative processes associated with prolonged HSF1 compromise.

An expanding array of small drug-like compounds is becoming available with the ability to limit heat-induced upregulation of HSP and other HSF1 targets in cells and animals (summarized in **Table 2**). These compounds have been studied most extensively for their ability to enhance the efficacy of hyperthermia treatment of cancer cells and tumor xenografts. Bioflavonoids such as quercetin have the longest history [69,70]. Phase I clinical trials of quercetin and a water soluble derivative of this compound confirmed that plasma concentrations consistent with those required to modulate the heat-shock response *in vitro* can be achieved with acceptable toxicity [71,72]. The activity of quercetin however is not specific to HSF1. At the concentrations required to inhibit the heat shock response, it also inhibits a range of protein kinases. This suggests a mechanism of action: quercetin might act by inhibiting a kinase required for HSF1 activation. Kinases are generally considered 'druggable' targets, making this an attractive starting point for further investigation, but low potency and lack of specificity limit enthusiasm for quercetin itself as a lead.

Synthetic benzylidene lactams such as KNK437 inhibit the heat-induced expression of HSP without decreasing basal levels of their constitutive isoforms [73]. These compounds have poor potency, but are relatively non-toxic. Their proximal target of action is unknown. Unlike quercetin, their mechanism does not appear to involve inhibition of HSF1 phosphorylation. The natural product stresgenin B has also been reported to inhibit heat-induced HSP gene expression, but its mode of action is uncharacterized [74].

Triptolide, acting in the low nanomolar concentration range, is the most potent inhibitor described to date. It does not inhibit HSF1 trimer formation, phosphorylation or tight DNA binding [75]. Instead it interferes with its transactivating activity by an as yet unknown mechanism; this activity too is not restricted to HSF1. Triptolide can also impair the transcriptional activity of NF-κB and activator protein 1 (AP-1) [76]. Although toxic at higher concentrations, a useable therapeutic index for triptolide in the treatment of pancreatic cancer xenografts has been demonstrated [77]. The extent to which the anticancer activity of triptolide results from disruption of HSF1 function is an important unanswered question.

Recently, a screen of 20,000 compounds for structures that block HSP induction identified 2 analogs of the general translational inhibitor dehydroemetine [78]. At low micromolar concentrations, these compounds (NZ28 and emunin) showed little acute toxicity, but sensitized cancer cells to the effects of HSP90 and proteasome inhibitors. Whether such sensitization might translate to improvement in the therapeutic index of these drugs or concomitantly increase their toxicity for normal tissues remains to be determined. The precise target of action for NZ28 and emunin is unknown, but seems to involve post-transcriptional events downstream of HSF1, leading to significant concerns over their specificity.

So far, no inhibitors of the heat shock response have yet been identified that directly target HSF1 and all inhibitors of HSP induction suffer from problems of low potency and/or poor specificity. This does not betoken poor progress or a fundamental barrier. Rather it reflects the limited resources and efforts that have been directed at this target to date. Powerful genetic tools have only recently revealed HSF1 as a key enabler of the malignant state [58]. The immediate challenge ahead is to make use of our new insights into HSF1 biology to devise effective strategies for the discovery of chemical biological probes with the requisite potency and specificity. Such compounds will feed back to further refine

Table 2. Drug-like inhibitors of the heat shock factor 1 (HSF1)-regulated heat-shock response.

Compound	Class	Ref.
Quercetin	Flavonoid	[69-71]
QC12	Quercetin prodrug	[72]
KNK437	Benzylidene lactam	[73]
Stresgenin B	Streptomyces fermentation product	[74]
Triptolide	Diterpene triexpoxide	[75-77]
Emunin	Emetine derivative	[78]
NZ28	Emetine derivative	[78]

our understanding of how HSF1 enables cancers and serve as leads for the development of useful drugs with which to exploit this intriguing 'non-oncogene' target.

5. Expert opinion

The HSF1-regulated heat shock response is a transcriptional program acting genome-wide to restore the normal protein folding environment and re-shape cellular pathways controlling apoptosis, proliferation and metabolism. Our recent work has shown that, surprisingly, the many beneficial effects of HSF1 in enhancing the survival of organisms under stress come at the cost of facilitating the initiation and maintenance of cancers. This has been demonstrated in a clinically relevant mouse model driven by activated oncogene as well as one driven by tumor suppressor mutation. Its relevance to human malignancy was established using diverse human tumor lines driven by a variety of underlying oncogenic lesions.

Acting at a global systems level, HSF1 function permits cells to survive the drastic imbalances in signaling and profound alterations in DNA, protein and energy metabolism that occur during malignant transformation. This deleterious effect of HSF1 arises as an unfortunate legacy of its ancient role in enhancing the survival of organisms exposed to a harsh and changing world, but it also suggests a unique therapeutic opportunity. Ablation of HSF1 expression using genetic techniques is well tolerated in normal cells and even whole animals under basal physiological conditions. That the malignant lifestyle confers a profound dependence on this 'non-oncogene' strongly supports the likelihood of an exploitable therapeutic index for inhibitors of HSF1 function.

The regulation of HSF1 activities is complex. Many questions remain at the molecular level despite considerable effort over the past three decades. Nevertheless, it is known that activation and subsequent de-activation of HSF1 involves a multi-faceted cascade of serine/threonine kinases and phosphatases. These should provide feasible, relatively 'drugable' targets for selective inhibition of HSF1 function and avoid the major problem for all small-molecule strategies of directly disrupting DNA–protein interactions. Interest in pharmacological manipulation of the heat shock response has already led to the empirical identification of several inducers and inhibitors of HSF1 activation. So far, none of these compounds seems to act upon HSF1 directly and all display prominent non-HSF1-dependent effects unique to their particular modes of action. In the case of HSP90 and proteasome inhibitors, for example, the cytoprotective activation of HSF1 occurs as a side-effect of their primary action and might impair their overall anticancer efficacy. Combined exposure to HSF1 inhibitors with diverse modes of action might increase their activity, but it might increase toxicity to normal tissues as well. The same concern applies to combination with non-specific cytotoxic agents. The combination of HSF1 inhibitors with highly selective compounds that target specific oncoproteins, however, might render agents such as kinase inhibitors more effective and reduce the frequent emergence of resistance.

Therapeutic induction of the multi-faceted HSF1-mediated stress response by non-cytotoxic exposure to HSP90 inhibitors and celastroloids is actively being studied in hypoxic-ischemic injury and protein misfolding disorders such as Huntington's, Alzheimer's and Parkinson's diseases. Whether prolonged HSF1 activation (multi-year time-frame) might increase the risk of oncogenic transformation is currently unknown, but of obvious concern. Conversely, inhibition of HSF1 activation provides a very promising new anticancer strategy and warrants vigorous discovery efforts. During development, however, attention will need to be given to the possibility that extended HSF1 inhibition (multi-month time-frame), especially by compounds that penetrate the CNS could exacerbate protein-aggregation-associated neurodegenerative disorders, especially in an older patient population.

Important questions remain, but there is cause for considerable enthusiasm. Given the wealth of basic scientific insights and the essential tools now in place, it is reasonable to expect that within the next 5 years potent and selective inhibitors of HSF1 will be identified and early pre-clinical evaluation of their anticancer activity will be well underway, alone and in combination with other agents.

Declaration of interest

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