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HSF1, a versatile factor in tumorigenesis

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Abstract

HSF1 is an essential factor in the acute response to proteotoxic stress, in which it causes rapid transcription of heat shock protein (HSP) genes in order to permit survival of cells and restoration of global protein quality. In addition to this property however, HSF1 is chronically activated or overexpressed in a wide range of cancers and is essential for multiple pathways of malignant transformation. Studies in recent years indicate a remarkable pleiotropy in the properties of HSF1 in cancer. HSF1 functions as a transcription factor for HSP genes, reminiscent of its role in the stress response, and the resultant elevation in HSP levels leads to a reduction in programmed cell death and senescence and permits overexpression of mutated oncogenic protein clients required to fuel tumor growth. In addition HSF1 plays a role as a signal modulator, stimulating kinase activity, regulating energy metabolism and permitting the development of polyploidy in cancer cells. HSF1 can also function as an inhibitor of transcription and in cooperation with NuRD family factors can repress genes that oppose metastasis. Inhibitors of HSF1 are undergoing selection and future studies may see the testing of HSF1 as a target in cancer therapy.

Introduction

Heat shock proteins (HSPs) are expressed abundantly after stresses such as heat shock in essentially all cellular organisms and mediate resistance to further stress [1, 2]. This universal response appears largely predicated on the ability of HSPs to deter unfolding of client proteins during stress and to mediate refolding [2, 3]. However, the response appears to have roles over and above surviving heat shock. The proteome is constantly vulnerable to protein stress due to sporadic mutations, oxidative stress and the expression of proteins with dominant aggregation prone conformations [4]. Protein stress is most pronounced in the central nervous system where neurons are relatively deficient in the heat shock response and a range of age associated protein folding disorders is observed [5]. Cancer, although another disease of advancing age involves the opposite scenario-elevated HSP levels that correlate with malignancy [6]. In neither case is the exact mechanism for the defect in HSP regulation completely understood. Expression of HSPs during the heat shock response depends on the transcription factor heat shock factor1 (HSF1) [7]. Factors involved in HSF1 regulation are emerging and characterizing the molecular control of this HSF1 may permit understanding of protein quality control pathways in stress and pathological conditions [6, 8].

(1) Current models of HSF1 regulation and signaling

The remarkable property of HSF1 activation by stress is its rapidity: activated HSF1 is found on the promoters of target genes within a few seconds of heat shock [9]. Indeed, HSP promoters appear primed for rapid activation and contain RNA polymerase II paused on the promoter, proximal to the transcription start site after transcribing approximately 20 base pairs [10]. The initial round of mRNA synthesis thus involves overturning the pause in transcription and rapid rounds of transcriptional elongation, preceding further rounds of initiation and elongation [9]. Key to the activation of HSP transcription is the association of HSF1 with the *positive translation elongation factor 1* (p-TEFb) which phosphorylates the c-terminal domain of RNA polymerase II, a key mechanism in the downstream steps of HSF1-mediated transcription [11]. A key question however, how is HSF1 activated within the cytoplasm?

Early studies indicated that HSF1 activation in stress is a posttranslational event [12]. Regulation of HSF1 during stress involves its escape from a largely inert complex containing monomeric HSF1 associated with a chaperone complex containing Hsp90 and a number of associated co-chaperones [13–15]. Consonant with its role in protein folding, activation of HSF1 involves a series of unfolding events, facilitated by the chaperone complex, leading to HSF1 activation [14, 15]. One hypothesis for HSF1 activation is that the chaperone complex becomes sequestered by proteins unfolded during stress and that HSF1 thus escapes the confines of the repressing complex [16]. Although compelling, this hypothesis does not explain the rapidity of the appearance of HSF1 on chromatin. Further hypotheses indicate a role for a large non-coding RNA species in stress activation, for deacetylation of HSF1 in the DNA binding domain as well as dephosphorylation of HSF1 at a site involved in Hsp90 binding [17–19]. HSF1 activation by stress may involve a constellation of these processes that work cooperatively.

The dominant paradigm for HSF1 activation by stress is that the event is wholly posttranslational [12]. However, this paradigm may not necessarily be adhered to in the case of HSF1 activation in cancer [20]. Studies of HSF1 levels in clinical breast cancer biopsies show increases in both mRNA and protein concentration that correlate with a bad prognosis [21]. In addition it has been shown that HSF1 is activated by the pro-malignant cytokine heregulin in breast cancer cells and that activation involves an increase in HSF1 levels as well as posttranslational modifications [21].

In addition to stress-induced activation of trimerization and nuclear localization, HSF1 contains a regulatory domain that appears to sense exposure to stress and controls HSF1 activity independently of DNA binding [22, 23]. Indeed this domain when fused to the activation domains of GAL4 can repress transcriptional activity in unstressed conditions and activate HSP gene transcription during exposure to stress [22, 23]. This domain contains multiple potential phosphorylation sites that can both reduce transcription and increase it [24]. The inhibitory functions of the regulatory domain may involve two closely apposed sites at serines 303 and 307 [24, 25]. Activation of the ERK pathway leads to phosphorylation on serine 307 which permits secondary phosphorylation at serine 303 by glycogen synthase kinase 3 (GSK3) [24, 25]. GSK3 represses HSF1 at least in part by

recruiting 14-3-3 molecules to HSF1-phospho-S303, S307 an event that leads to nuclear exit and removal from HSP promoters [26, 27]. This pathway is also involved in HSF1 regulation in breast cancer. Treatment of mammary cells with heregulin causes HSF1 activation and HSP expression [20]. This pathway is mediated at least partially through the S303 phosphorylation site on HSF1. Heregulin binding triggers a pathway leading to activation of HER2 kinase on the mammary cell surface and a cascade response involving downstream induction PI-3 kinase and Akt kinase [20]. Activation of Akt then leads to inhibitory phosphorylation on GSK3, an event that leads to de-repression of HSF1. We have recently discovered an activating phosphorylation site within the regulatory domain of HSF1, at serine 320 [28]. S320 is phosphorylated by protein kinase A (PKA) after both heat shock and heregulin treatment [28]. Thus heregulin leads to HSF1 activation both by inhibiting repressive phosphorylation and by inducing at least one positively acting phosphorylation event at serine 320. In addition, as mentioned above, HSF1 levels increase in breast cancer cells and elevated HSF1 concentrations are observed in clinical samples of advanced breast cancer [20, 21, 29]. Thus HSF1 may undergo enhanced expression in cancer and this effect is likely to increase its ability to activate transcription (Fig. 1).

(2) HSF1 as a signal modulator

Recent studies indicate that HSF1 is essential for malignant transformation by multiple carcinogenic stimuli including: chemical carcinogens, ablation of intracellular Tp53, Ras expression and exposure to cytokines such as platelet derived growth factor and heregulin [30, 31]. The exact role of HSF1 in transformation is not clear although it may involve the ability of the factor to foster multiple rounds of mutation in the evolving tumor and permit the appearance of new malignant phenotypes (reviewed recently): [6]. Another property of HSF1, that is not directly related to its transcription factor properties, is the ability to modulate a range of signal transduction pathways. HSF1 has been shown to bind to a number of protein kinases [27, 28, 32, 33]. These include the catalytic domain of DNA-dependent protein kinase, a molecule involved in the DNA damage response pathway that is activated upon HSF1 binding, and further stimulated by association with heat shock elements [33]. In addition, HSF1 can bind to extracellular signal activated protein kinase (ERK) in cancer cells leading to phosphorylation of HSF1 and potentially other substrates. We have shown recently that HSF1 binds stably to the catalytic domain of protein kinase A, promotes its nuclear localization and activates its transcription. HSF1 appears to be required for PKA activity in cancer cells and phosphorylation of exogenous PKA substrates is inhibited by HSF1 knockdown [28]. HSF1 thus appears to be both a substrate for PKA and a factor stimulating PKA activity in malignant cells [28]. We have shown that treatment of mammary carcinoma cells with heregulin leads to enhanced association of HSF1 with PKA and elevated HSP synthesis [28]. HSF1 may thus be able to bind to the catalytic domains of kinases and direct them to substrates important in malignant transformation.

(3) HSF1, polyploidy and the cell cycle

One of the original mechanisms shown to be regulated by HSF1 in cancer is generation of polyploidy in prostate carcinoma [34]. Polyploidy is a feature of many cancers and leads to genomic instability and tumor progression [35, 36]. We have shown that antagonism of HSF1 activity in PC-3 prostate carcinoma cells using expression of a dominant negative

construct leads to a reduction in polyploidy and return to diploid DNA content, while increased expression of HSF1 had the opposite effect [34]. HSF1 therefore appears to foster the development of polyploidy, and it is notable that PC-3 cells express abundant HSF1 levels [37]. Polyploidy is caused by a breakdown in the mitotic spindle assembly checkpoint, a mechanism in which feedback between DNA replication and cell division normally occurs [35, 36]. Polyploidy is often seen in cells in which p53 is inactivated: p53 regulates the tetraploidy pathway, another mechanism for avoiding polyploidy [38]. However, we have shown that HSF1 operates independently of p53 and promotes polyploidy in p53 deficient cells [34]. Elevated levels of HSF1 thus appear to override the mitotic spindle assembly checkpoint and permit emergence of polyploidy in prostate carcinoma. This checkpoint is regulated by cyclin B1, a protein that is required to be degraded before mitosis can be completed. In cells with normal wild-type p53 content, replication cannot commence until cell division is completed [38]. Expression of dominant negative HSF1 delays the decay in cyclin B1 and this mechanism may contribute to its role in enhancing aneuploidy [34]. Recent studies have identified a mechanism for degradation of cyclins and other proteins involved in mitosis involving the anaphase promoting complex and ubiquitin E3 ligase Cdc20 [35]. We have found that Cdc20 binds directly to HSF1 and this interaction may thus be involved in overriding the mitotic spindle checkpoint (R. Zhong & S.K. Calderwood, in preparation).

(4) HSF1, HSPs and programmed cell death in cancer

Reduction in levels of programmed cell death such as caspase dependent apoptosis is a general property of malignant cells and contributes to treatment resistance [39]. Loss of vulnerability to apoptosis may protect tumor cells in hostile, unperfused environments, and allow metastatic cells to survive in the circulation. In addition, resistance to apoptosis may be required during transformation as normal cells possess an in-built response in which expression of oncogenes such as Myc leads to programmed cell death [39]. We have shown that induction of HSF1 activity leads to protection of breast cancer cells from apoptotic killing and that mouse embryo fibroblasts (MEFs) with HSF1 inactivation are sensitive to killing by chemotherapeutic agents [20]. Likewise, reduction in HSF1 levels in pancreobiliary cancers led to increase in annexin V, TUNEL positivity and caspase-3 activation and decreased cell death (Dudeja 2011). These effects likely involve the elevation in Hsp27 and Hsp70 that occurs in breast cancer and a wide range of other malignancies [40]. Elevated levels of Hsp27 and Hsp70 mediate resistance to apoptotic death [41]. In addition, it was shown that HSF1 protects colon cancer cells from apoptosis through a pathway involving induction of the co-chaperone BAG-3 which appears necessary for expression of the Bcl2 family of anti-apoptotic proteins [42]. HSF1 may also inhibit apoptosis through its ability to repress transcription. HSF1 represses the expression of XAF1 (XIAP-associated factor) in gastrointestinal cancers and relieves the inhibition of activity of the anti-apoptotic mediator XIAP [43]. These effects appear to be important components of the stress response, hijacked in cancer and mediated by direct HSP binding to multiple apoptotic regulators. The apoptotic cascade thus appears to become jammed at multiple points when HSF1 and HSP levels become elevated in cancer. The mechanisms involved in apoptosis inhibition by the HSPs are not entirely clear. However, binding to Hsp70 is one of the early stages in the molecular chaperone cascade, involved with the initial stages of

folding [3]. Association of apoptotic regulators with Hsp70 may thus hold the proteins in inactive conformations incapable of mediating cell death. Down-regulation of the apoptotic cascade would permit emergence of the malignant phenotype and protect cancer cells from therapies that operate through stimulation of apoptotic killing

Another pathway of cancer cell inactivation is through senescence [44]. Senescence occurs in normal cells that have exceeded a limiting number of cell divisions. Cell divisions are “counted” by a molecular mechanism involving the telomere structures on the ends of chromosomes [45, 46]. During cell division, the telomeres shorten until reaching a limiting length at which point the senescence mechanism is triggered. A number of recent studies from the Sherman lab indicate that, as with apoptosis, elevated levels of Hsp27 and Hsp70 can inhibit senescence and aid in the emergence of breast cancer [47–49]. In addition, a number of approaches to therapy inactivate cells through a senescence-type mechanism and cancer cells expressing elevated levels of HSPs may thus acquire treatment resistance through this pathway [50]. Thus Hsp27 and Hsp70 play an important role in transformation and tumor formation by inactivating both apoptosis and senescence in the emerging cancer cell. It has also been shown that elevated levels of Hsp70 can inhibit unconventional pathways of cell killing involving lysosomal hydrolases, indicating the versatility of HSPs in evading tumor cell death [51].

(5) HSF1, the epithelial-mesenchymal transition (EMT) and metastasis

Two of the stages in tumor progression associated with particular morbidity are cancer cell invasion and metastasis. Elevated expression of HSF1 has been associated with advanced stages in breast cancer, hepatocellular carcinoma and oral squamous cell carcinoma, suggesting a broad role for the factor in these processes [21, 52–54]. It seems likely that the ability of HSF1 to promote cancer cell signaling, to enhance polyploidy and evade cell inactivation pathways likely contribute to tumor progression as well as initiation. However, other properties of HSF1 may be important in promoting these effects. Both metastasis as well as ability to invade the extracellular spaces and enter the microcirculation is associated with a change in phenotype known as the epithelial-mesenchymal transition (EMT), which involves a profound remodeling in gene expression as well as morphology [55]. We have found that HSF1 overexpression in mammary carcinoma leads to an EMT phenotype and ability to grow under anchorage independent conditions, a known characteristic of cells that have undergone the EMT [29]. Similar effects of acquisition of an EMT phenotype were observed with an important mediator of metastasis- metastasis-associated protein 1 (MTA1). This protein has the function of a gene co-repressor and has been closely associated with the metastatic phenotype in mammary cancer. We have found that HSF1 is required for MTA1 expression in mammary cancer and can subsequently bind with HSF1 in a co-repressing complex [29]. Previous studies indicated that one of the properties of MTA1 in metastatic mammary carcinoma is to repress the transcription of estrogen receptor- regulated genes [56]. Estrogen mediated transcription through estrogen receptor (ER) binding to its targets in the genome leads to mammary cancer growth. However, progression to the metastatic phenotype involves the HER2 pathway which can subdue estrogen/ER mediated transcription [56]. We have found that ER-mediated transcription and gene expression can be inhibited by either HER2 activation by heregulin, MTA1 overexpression or forced HSF1

expression in mammary carcinoma cells. In addition, HSF1/MTA1 complexes were observed at ER binding *loci* including the *Myc* and *Psn2* genes in ER responsive mammary carcinoma cells [29]. HSF1 and MTA1 are also co-overexpressed in the nuclei of advanced, metastatic mammary carcinoma studied in archived biopsy samples [29]. HSF1 may therefore play a key role in the EMT transition, invasion and metastasis (Fig 2).

(6) HSF1 and the intermediary metabolism of cancer cells

Cancer is associated with profound alterations in intermediary metabolism. This is one of the earlier findings in study of the pathophysiology of cancer and involves the ability of cells to metabolize glucose via glycolysis in the presence of O₂ (oxidative glycolysis) [57]. This property results in avid uptake and utilization of glucose and a tumor milieu low in glucose and enriched in metabolic acids [58]. A number of studies link HSF1 to an altered metabolism in a cancer. Dai et al showed a profound decrease in glucose uptake in tumor cells with HSF1 inactivation [30]. In addition HSF1 appears to function downstream of HER2 in modulating glucose metabolism and increasing the rate of glycolysis [59]. HSF1 was required for HER2 dependent expression of the key glycolysis enzyme lactate dehydrogenase (LDH-A). Thus HSF1 may participate in the malignant phenotype in regulating linked uptake of glucose and its metabolism through glycolysis [59]. HSF1 also appears to mediate development of hepatocellular carcinoma (HCC) by decreasing insulin sensitivity through effects on metabolism [53]. HSF1 knockdown decreased HCC progression by decreasing fat metabolism and accumulation and decreasing insulin resistance. These effects were linked to the protein kinase AMP-K[53]. HSF1 inactivation increased levels of AMPK and activity an effect linked to decreased fat metabolism in the absence of the factor (Fig 2).

(7) Targeting HSF1 with chemical inhibitors- theory and practice?

It would seem therefore that targeting HSF1 with small molecule inhibitors might be a viable approach to cancer therapy. A number of inhibitors have been found to block HSF1 activity, although convincing preclinical data on their utility is not available. Initial studies employed tyrosine kinase inhibitors such as genistein and quercetin that block an upstream step in HSF1 activation. Although HSF1 is not directly phosphorylated on tyrosine, the inhibitors can effectively block the response of HSF1 to stress, presumably by blocking an upstream kinase [24, 60]. Quercetin has been shown to have some effectiveness in treating prostate cancer in a preclinical animal model [61]. Questions remain however regarding the specificity of this drug for HSF1. Other HSF1 targeting drugs have also been selected, including emunin, a drug that prevents HSP synthesis and sensitizes cells to Hsp90 inhibitors and proteasome inhibitors [62]. The drug was not however highly effective in killing target cells as a single agent. More recently the diterpine triepoxide drug triplotide was shown to inhibit HSP gene transcription, inhibit HSF1 and sensitize cells to stress-induced death [63]. However this agent does not appear specific for HSF1 and also inhibits the transcription factors NF- κ B and AP-1 [63]. Finally, another agent that has recently emerged is KRIBB11 (N2-1H-indazole-5-yl)-N6-methyl-3-nitropyridine-2,6-diamine), a compound that binds HSF1 directly and inhibits tumor growth [64]. These investigators show that KRIBB11 inhibits recruitment of p-TEFb by HSF1. It will be interesting to examine whether the available HSF1-targeting compounds are fully specific, non-toxic in

normal tissues, have solubility properties suitable for *in vivo* use and can function as single chemotherapy agents in cancer therapy. In theory effective HSF1 drugs might be expected to surpass the potency of Hsp90 inhibitors, due to the position of HSF1 at the head of the molecular chaperone cascade and its other properties in tumorigenesis. The next few years may bring us answers to these questions.

Conclusions

Recent studies indicate a profound role for HSF1 in the development of multiple cancer cell types. These effects include: the promotion of cell survival, overturning cell cycle checkpoints, and mediation of tumor progression and energy metabolism. These pleiotropic and essential properties in carcinogenesis and tumor progression indicate that HSF1 may be a promising target in therapy. A number of drugs that inhibit HSF1 activity have been synthesized in recent years and future studies may indicate whether effective compounds, with suitable *in vivo* properties, can be made and deployed in cancer therapy.

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Figure 1. HSF1 becomes activated in cancer

Multiple mechanisms may be involved in activation, including increased *de novo* synthesis of HSF1, posttranslational mechanisms including coupling to malignant cell signaling such as the HER2 pathway or de-repression of HSF1 due to the proliferation of Hsp90 clients (overexpressed mutant oncogenes) and escape from control by Hsp90.

Figure 2. Versatile downstream effects of HSF1 after activation in cancer development

Some of the effects mediated by active HSF1 likely involve: (1) Elevated synthesis of HSPs and increased chaperoning power in the cells as well as decreased sensitivity to cell death pathways. (2) HSF1 also plays a currently ill-defined role in cell signaling and may thus influence cell cycle progression and energy metabolism in the tumor cells. (3) HSF1 is effective in repressing multiple non-HSP genes and may play a role in the gene expression alterations that accompany development of a metastatic phenotype and maintain cancer stem cell pluripotency.