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Proteotoxic stress of cancer: implication of the heat-shock response in oncogenesis

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Abstract

Organisms frequently encounter a wide variety of proteotoxic stressors. The heat-shock response, an ancient cytoprotective mechanism, has evolved to augment organismal survival and longevity in the face of proteotoxic stress from without and within. These broadly recognized beneficial effects, ironically, contrast sharply with its emerging role as a culprit in the pathogenesis of cancers. Here, we present an overview of the normal biology of the heat-shock response and highlight its implications in oncogenic processes, including the proteotoxic stress phenotype of cancer; the function of this stress response in helping cancer survive and adapt to proteotoxic stress; and perturbation of proteome homeostasis in cancer as a potential therapeutic avenue.

Keywords

Oncogenesis; Proteotoxic stress; Heat-shock response; HSF1

Cancer is the second most common cause of death; over half a million Americans die from cancer each year. Despite the tremendous efforts in cancer research and treatment over the past four decades, progress has been frustratingly slow. It is clear that we are far from winning the War on Cancer.

The development of cancer, or oncogenesis, is a dynamic multi-step process that involves the initiation, promotion/maintenance, and progression stages. The complexity of human cancer is overwhelming, reflected by vast genetic and epigenetic diversity, remarkable cellular heterogeneity, and phenomenal tumor evolvability. Given this daunting complexity, targeting cellular pathways that are universally utilized by cancer cells but not by their normal counterparts represents a potentially promising strategy. The latest developments in cancer research suggest that the heat-shock response may be one such key pathway.

THE BIOLOGY OF THE HEAT-SHOCK RESPONSE

The guardian of proteome homeostasis

Organisms commonly encounter a wide variety of environmental insults. To sustain the cellular homeostasis that is vital to the normal functions of cells and fitness of organisms, certain cellular programs promptly respond to various stressors. Responses include the heat-shock, or stress, response, one of the most ancient and evolutionarily conserved cytoprotective mechanisms found in nature (Ritossa, 1996). Following exposure to environmental insults, the cells in most tissues dramatically increase the production of a group of proteins that are collectively known as “heat-shock” or stress proteins (HSPs)

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(Lindquist, 1986; Morimoto et al., 1997). These include several conserved classes based upon their molecular weights: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs. These stress proteins perform multiple functions that protect cells from stress, and their drastic induction has become the unique hallmark of the heat-shock response.

Work by many groups over the past 30 years has revealed that HSPs and their close, constitutively expressed relatives function as “molecular chaperones”, proteins that guard against “illicit or promiscuous interactions” between other proteins (Craig et al., 1993; Benjamin and McMillan, 1998). These chaperones protect the proteome from the dangers of misfolding and aggregation by facilitating protein folding, trafficking, complex assembly, and ubiquitination, as well as proteasomal degradation (Balch et al., 2008; Morimoto, 2008). Their increased expression in tissues that are subjected to various proteotoxic stressors including heat, heavy metals, UV radiation, hypoxia, desiccation, and acidosis represents an adaptive response that has proven to be essential to proteome homeostasis and cell survival. Many of these stress conditions are, in fact, highly relevant to the pathophysiology of common human diseases.

Heat shock factor 1 (HSF1) and the heat-shock response

A small group of transcription factors called heat shock factors (HSFs) regulate the heat-shock response. In contrast to a single HSF in yeasts, worms and flies, four HSFs—HSF1, 2, 3 and 4—have been identified in mammals that play a role in transcriptional control of HSP gene expression under stress conditions (Akerfelt et al., 2007; Fujimoto et al., 2009). All HSFs share structurally conserved domains, of which the most preserved is the N-terminal helix-turn-helix DNA-binding domain (DBD) (Akerfelt et al., 2007). All HSFs bind *via* their DBD to consensus Heat Shock Elements (HSE), consisting of multiple adjacent inverted arrays of the binding site (5'-nGAAn-3'), within the promoter regions of the known HSP genes (Akerfelt et al., 2007). Although HSF2, HSF3, and HSF4 are implicated in modulating this stress response, HSF1 has clearly emerged as the dominant factor controlling cellular responses to heat and many other proteotoxic stressors (Ankar and Sistonen, 2011). For example, genetic knockout of *Hsf1* in mammalian cells is sufficient to completely abrogate induction of the stress response following heat shock (Xiao et al., 1999; Zhang et al., 2002). In sharp contrast, cells deficient for HSF2, HSF3, or HSF4 retain an intact stress response (McMillan et al., 2002; Fujimoto et al., 2004; Fujimoto et al., 2009).

Mechanisms of HSF1 activation

The activation of HSF1 triggered by stresses is a multi-step process. Under non-stressed conditions, HSF1 remains in a dormant state as inactive monomers by forming protein complexes with chaperones/co-chaperones including HSP90, HSP70, HOP, and others (Wu, 1995; Ankar and Sistonen, 2011). These repressive complexes dynamically shuttle between the cytoplasm and nucleus. Activation of HSF1 takes place via multiple alternative mechanisms. According to the unfolded protein titration mechanism, chaperones within the repressive HSF1-containing multichaperone complexes, such as HSP90 and HSP70, are rapidly titrated away following heat or other stresses by a large pool of unfolded proteins. Following dissociation of the repressive complexes, monomeric HSF1s undergo trimerization and subsequent nuclear localization (Wu, 1995; Ankar and Sistonen, 2011). Nuclear HSF1 trimers acquire DNA binding capability and transcriptional competence (Voellmy, 2004).

HSF1 can also be activated by a ribonucleoprotein complex. It was reported that during heat stress, trimerization and DNA binding of HSF1 are enhanced by a ribonucleoprotein complex that includes heat shock RNA-1, a constitutively expressed non-coding RNA, and EEF1A, a translation elongation factor (Shamovsky et al., 2006). In addition, HSF1 can

directly sense stresses through two cysteine residues located within its DNA-binding domain (Ahn and Thiele, 2003).

Following binding to DNAs, trimeric HSFs fully engage in transcriptional activation upon further posttranslational modifications such as phosphorylation. Both stimulatory and inhibitory phosphorylation have been documented. For example, phosphorylation of Ser230 and Ser326 markedly promotes HSF1 transcriptional activity (Holmberg et al., 2001; Guettouche et al., 2005). In contrast, constitutive phosphorylation of Ser303, Ser307, and S363 imposes an important negative regulation under normal growth conditions (Park and Liu, 2001; Tang et al., 2001). Several kinases have been implicated in phosphorylating HSF1. Extracellular signal-regulated kinase 1/2 (ERK1/2) and glycogen synthase kinase 3 (GSK3) negatively modulate HSF1 activity by phosphorylating Ser307 and Ser303, respectively (Tang et al., 2001). In contrast, calcium/calmodulin-dependent kinase II (CaMKII) promotes HSF1 activity by phosphorylating Ser230 (Holmberg et al., 2001). Casein kinase 2 (CK2) also positively modulates HSF1's thermal activation, by phosphorylating Thr142 (Soncin et al., 2003). Furthermore, polo-like kinase 1 (PLK1) positively modulates HSF1 activity, by phosphorylating Ser419, a process that regulates HSF1's nuclear translocation (Kim et al., 2005).

HSF1 undergoes other forms of posttranslational modifications in addition to phosphorylation. Sumoylation of HSF1 on Lys298 upon stresses requires prior phosphorylation of Ser303 and leads to repression of HSF1 transcriptional activity (Hietakangas et al., 2003). Stresses also induce acetylation of HSF1 on lysine residues, which appears to be involved in attenuation of the heat-shock response (Westerheide et al., 2009). Intriguingly, SIRT1, a key metabolic sensor and longevity factor, can enhance the heat-shock response by directly deacetylating HSF1 (Westerheide et al., 2009).

Complex gene regulation network of HSF1

In a role much less appreciated than classic HSP induction, the heat-shock response coordinates global, well-integrated alterations of cellular physiology to help organisms effectively cope with deleterious environmental fluctuations, both external and internal. Congruously, recent genome-wide studies have revealed previously unappreciated complex regulatory networks of HSF1 beyond HSPs induction. For example, surprisingly, in yeast HSF1 directly regulates the expression of up to 3% of the whole genome and these target genes are involved in a broad range of cellular functions including energy production, signal transduction, small-molecule transport, carbohydrate metabolism, cytoskeletal organization, and vesicular transport (Hahn et al., 2004). A similar scenario is being uncovered in *Drosophila* and mammals (Trinklein et al., 2004; Birch-Machin et al., 2005). Individual laboratories have already demonstrated that HSF1, acting as either a transactivator or a repressor, regulates the expression of numerous non-HSP genes with diverse functions including interleukin 1 β (IL-1 β) (Xie et al., 2002), interleukin 6 (IL-6) (Inouye et al., 2004), c-fos (Xie et al., 2003), c-mos (Xie et al., 2003), XIAP associated factor 1 (XAF1) (Wang et al., 2006), BCL2-associated athanogene 3 (BAG3) (Franceschelli et al., 2008), clusterin (CLU) (Michel et al., 1997), thrombomodulin (THBD) (Fu et al., 2008), heme oxygenase 1 (HO-1) (Koizumi et al., 2007), and plasminogen activator inhibitor-1 (PAI-1) (Zhao et al., 2008). In fact, this pleiotropic action of HSF1 befits its emerging role as a central coordinator of an extensive array of cellular pathways upon stress, which allows cells to survive and efficiently adapt to harmful conditions.

The heat-shock response and diseases

Although dispensable for growth and survival under normal conditions, the HSF1-mediated stress response is crucial for cells to survive stresses from without and within. It is known

that this stress response and individual HSPs protect cells from a broad range of pathological insults including hyperthermia, heavy metal toxification, ischemia/reperfusion, oxidative damage, chronic inflammation, and neurodegeneration (Westerheide and Morimoto, 2005). For example, activation of HSF1 protects mouse cardiomyocytes from death following ischemia/reperfusion injury. HSF1 also rescues neurotoxicity and prolongs lifespan in a number of neurodegenerative disease models including polyglutamine disease, Parkinson's disease, and prion disease (Fujimoto et al., 2005; Shen et al., 2005; Steele et al., 2008). In a mouse model of inflammatory bowel disease (IBD), HSF1 is shown to protect against colitis by suppressing the expression of pro-inflammatory cytokines and reactive oxygen species (ROS)-induced cell death (Tanaka et al., 2007). In stark contrast, the impact of the HSF1-mediated stress response on cancers has remained largely unclear.

THE HEAT-SHOCK RESPONSE IN ONCOGENESIS

HSPs and cancer

Elevated expression of individual HSPs has been observed in a wide array of human cancers (Calderwood et al., 2006; Sherman and Multhoff, 2007). HSPs can form complexes with and stabilize various oncogenic proteins, such as BCR-ABL, mutant TP53, mutant BRAF, AKT, Cyclin D1, CDK4, and ErbB2/HER2 (Dai and Whitesell, 2005; Solit et al., 2005). In addition, HSPs can suppress cell death by interacting with key apoptotic molecules, such as APAF1 and caspase 3 (Beere et al., 2000; Concannon et al., 2001). Thus, in cancer cells, increased expression of individual HSPs reinforces these oncogenic events and/or supplies the cooperative factors. However, until recently it remained elusive whether cells endure proteotoxic stress from within during malignant transformation and whether the heat-shock response plays a role as a systemic cellular adaptation in this process.

HSF1 and oncogenesis

Our and others' studies provided compelling evidence that the HSF1-mediated stress response is a powerful enabler of tumorigenesis. A recent study of ours indicated that genetic elimination of *Hsf1* in mice potently suppresses *in vivo* tumor formation initiated either by activation of the *Ras* oncogene in the classic chemical-induced skin carcinogenesis model or by inactivation of the tumor suppressor gene *Trp53* in a model of Li-Fraumeni syndrome (Dai et al., 2007). The action of HSF1 is pleiotropic, as HSF1 promotes cell proliferation and/or cell survival in response to diverse oncogenic stimuli, enhances ERK activation in response to serum stimulation, modulates protein translation, particularly the mammalian target of rapamycin (mTOR) pathway, and supports glucose uptake and glycolysis (Dai et al., 2007). Thus, it appears that HSF1 enables robust cellular transformation by orchestrating a network of core cellular functions including cell proliferation, survival, signal transduction, and metabolism.

Furthermore, our studies demonstrated that HSF1 maintains the malignant phenotypes of established cancer cells. *In vitro* HSF1 inhibition by small hairpin RNAs (shRNAs) impairs the growth and survival of a collection of human cancer cell lines that are derived from diverse histological origins and harbor a broad range of molecular defects, but has little or no effect on their non-transformed counterparts (Dai et al., 2007). In a genetically defined human mammary epithelial cell system, HSF1 dependence appears to correlate positively with cellular malignant state (Dai et al., 2007).

In a model system of human Neurofibromatosis Type I (NF1), we further confirmed and extended our initial findings on the role of HSF1 in potentiating tumorigenesis (Dai et al., manuscript submitted). Loss of *Hsf1* significantly prolongs the tumor-free survival of mice deficient for both the *Nf1* and *Trp53* tumor suppressor genes. At the molecular level, in the same study we uncovered an intriguing feed-forward loop between the oncogenic MAPK

signaling pathway and HSF1 activation. We showed that hyper-activation of ERK/MAPK signaling due to NF1 loss activates HSF1 by enhancing its phosphorylation as well as its nuclear translocation. In turn, HSF1 robustly supports increased flux through this signaling axis in part by stabilizing the key scaffolding protein Kinase Suppressor of RAS 1 (KSR1). Our data further indicated that HSF1 protein levels are markedly increased in primary human NF1 tumor tissues compared with their neighboring normal nerve tissues. Consequently, we demonstrated that tumor cell lines derived from human NF1 patients become reliant on HSF1 for their growth and survival.

A rapidly growing number of reports from other groups also strongly support a critical role of HSF1 in oncogenesis. It has been shown that HSF1 activation is important to maintain certain malignant phenotypes driven by oncogenic heregulin beat1, including anchorage-dependent growth and resistance to apoptosis (Khaleque et al., 2005). In addition, in aggressive breast tumors HSF1 is required for the cell transformation and tumorigenicity induced by the human epidermal growth factor receptor-2 (HER2/ERBB2) oncogene (Meng et al., 2010). Interestingly, a recent study indicated that HER2/ERBB2 activates HSF1 to promote glycolysis and growth of breast cancer cells by upregulating lactate dehydrogenase A (LDH-A) expression (Zhao et al., 2009). In a study integrating genetically engineered mouse models, cross-species cancer genomics, and functional screens, HSF1 was identified as a potent proinvasion oncogene in human melanomas (Scott et al., 2011). Consistent with this, silencing of HSF1 by shRNAs impairs proliferation of human melanoma cell lines (Nakamura et al., 2010). Also, suppression of HSF1 by siRNAs induces cell death in human pancreatic and cholangiocarcinoma cell lines and impairs cell proliferation in human leukemia cells expressing the FGFR1OP2-FGFR1 oncoprotein (Dudeja et al., 2011; Jin et al., 2011). In mice, *Hsf1* deficiency suppresses lymphomagenesis initiated by loss of *Trp53* as well as development of hepatic steatosis and hepatocellular carcinoma initiated by procarcinogen diethylnitrosamine (Min et al., 2007; Jin et al., 2011). Mechanistically, HSF1 can mitigate adverse effects of carcinogens on hepatic metabolism in mice by enhancing insulin sensitivity and sensitizing activation of AMP-activate kinase (AMPK) (Jin et al., 2011).

HSF1 expression in human cancers

Consistent with the above findings, aberrant HSF1 expression has been evidenced in a range of primary human cancer tissues. It was reported that HSF1 protein is overexpressed in malignant prostate epithelial cells relative to normal non-transformed prostate cells (Hoang et al., 2000). In human pancreatic cancer tissues, HSF1 expression is markedly increased compared to normal pancreatic tissues from the margin (Dudeja et al., 2011). In human hepatocellular carcinomas, HSF1 gene copy number is increased compared to normal liver tissues, and high expression levels of HSF1 are correlated with high Edmondson-Steiner grades as well as poor overall survival in patients (Woo et al., 2009; Fang et al., 2011). Similarly, human mycosis fungoides, the most common cutaneous T-cell lymphoma, frequently exhibit HSF1 gene amplification that is associated with patients' poor prognosis (van Doorn et al., 2009). In primary oral squamous cell carcinomas, HSF1 mRNA expression is significantly greater compared with expression in their normal counterparts, and higher nuclear HSF1 expression is closely related to tumor size and histopathologic type (Ishiwata et al., 2011). In a study of over 1,800 women with invasive breast cancer, HSF1 activation is shown to be an independent predictor of poor outcome (Santagata et al., 2011). In this large cohort study HSF1 expression is not only associated with high histologic grade, larger tumor size, and nodal involvement at diagnosis but also independently associated with increased mortality in the estrogen receptor-positive population.

Collectively, the accumulating evidence pinpoints a pivotal role of HSF1 and its mediated stress response in oncogenic processes. Moreover, the evidence indicates that involvement of this stress response pathway in human cancers is likely broad, if not universal.

PROTEOTOXIC STRESS AND THE ADAPTATION OF CANCER

Conceptually, these findings bring forth a new paradigm of carcinogenesis. In this paradigm, malignant transformation, or tumor initiation, is a “stressful” process accompanied by substantial disturbance in the delicate proteome homeostasis inside cells (Fig. 1). During cellular transformation numerous inherent factors contribute to the disruption of proteome homeostasis and instigation of proteotoxic stress, including dysregulation of the protein translation machinery; imbalanced protein production due to aneuploidy and gene amplification; accumulation of mutated, highly chaperone-dependent oncoproteins; and escalation of protein damages due to oxidative stress (Workman, 2003; Davies et al., 2005; Williams and Amon, 2009; Silvera et al., 2010). Most cancer cells, regardless of the genetic and epigenetic abnormalities they have, are likely under some degree of proteotoxic stress. To counteract this, the multifaceted heat-shock response is elicited to help cells undergoing malignant transformation to survive the internal stresses from within.

Further, these findings suggest that the HSF1-mediated stress response, dispensable for normal cells, remains activated in established cancer cells even in the absence of environmental challenges, and becomes fully integrated into their malignant lifestyle. Thus, malignant state continually creates proteotoxic stress, and established cancer cells likely have become dependent on this stress response to restrain proteomic disturbances within levels essential for maintaining their optimal fitness and expressing various malignant phenotypes. Unlike “oncogene addiction”, in which the dependence is limited to particular oncogenic events (Sharma et al., 2007), addiction of cancer cells to the HSF1-mediated stress response would be dictated only by the disturbed proteome homeostasis or malignant state of cells, and by environmental cues. Regrettably, cancer cells hijack and exploit this potent adaptive mechanism to survive and prosper, and to shape the ultimate landscape of malignancy.

THERAPEUTIC PERTURBATION OF PROTEOME HOMEOSTASIS IN CANCER

Of note, the extraordinary genetic and epigenetic diversity of human cancers poses a grave barrier to current therapies. While future personalized medicine aims to address this grand challenge (Sikora, 2007; van’t Veer and Bernards, 2008), our and others’ studies suggest that the presence of proteotoxic stress likely represents a widespread hallmark and vulnerability of cancer cells that could be readily exploited to combat human malignancies. This “stress phenotype” of cancer may serve as a generic prognostic marker for human cancers. Moreover, therapeutically disrupting the already strained proteome homeostasis in cancer cells may be of therapeutic value.

Disruption of proteome homeostasis can be accomplished by either exacerbating intrinsic proteotoxic stress, diminishing cellular defense mechanisms, or both in combination. On the one hand, cancer cells can be overburdened with excessive proteotoxic stress caused by accumulation of ubiquitinated or misfolded proteins, such as impairment of proteasome or chaperone functions. In fact, small molecule inhibitors of both the proteasome and HSP90 are currently in clinical trials for treatment of particular types of human cancers (Cusack, 2003; Workman et al., 2007). Agents affecting protein folding within the Endoplasmic Reticulum (ER) compartment and eliciting ER stress also fall into this category. On the other hand, genetic or chemical approaches can be applied to suppress the HSF1-mediated

stress response that remains constitutively active within cancer cells. Highly effective RNAi reagents have been reported by us and others that can markedly deplete cellular HSF1 levels (Dai et al., 2007; Nakamura et al., 2010; Dudeja et al., 2011; Jin et al., 2011). Unfortunately, no small, drug-like inhibitors of the heat-shock response have yet been identified that target HSF1 in a convincingly specific manner. Most, if not all, appear to act on the cascade of post-translational modifications mediated by kinases, phosphatases, acetylases and conjugating enzymes involved in regulating HSF1 activation (Yokota et al., 2000; Westerheide et al, 2006; Yoon et al., 2010). Consequently, promiscuous off-target effects may limit the clinical development of such new inhibitors reported to date; however, discovery efforts are in their infancy, with great potential for further progress.

CONCLUSIONS

As a nascent research area, the role of HSF1 and its mediated stress response in oncogenesis is largely unclear, with many fundamental questions remaining that warrant in-depth interrogations. While its importance to cancer has become increasingly apparent, little is known about the precise mechanisms underlying the activation and action of this powerful adaptive cellular program in oncogenesis. For example, during malignant transformation, what are the intrinsic signals that trigger HSF1 activation in the absence of environmental insults? What posttranslational modifications are required to fully engage HSF1? At the molecular level, what target genes are regulated by HSF1 in cancer cells? And, are these target genes the same as those regulated under classical proteotoxic insults? By what means does the HSF1-mediated stress response maintain the vigorous malignant phenotypes of cancer cells? Furthermore, it remains elusive whether and how this stress response facilitates the progression of cancer. Regarding potential clinical applications, do HSF1 expression and activation faithfully reflect tumor malignancy? Ultimately, how effective is targeting the HSF1-mediated stress response as an anti-cancer therapy?

Answering these questions will not only help expand and deepen our knowledge of cancer biology but also provoke innovative thinking about human cancer treatment.

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Fig 1. Cancer cells endure internal proteotoxic stress

Oncogenic signaling enhances protein synthesis by hyperactivating cellular protein translation machinery. In addition, oncogenic signaling causes dysregulation of mitochondrial metabolism and overproduction of reactive oxygen species (ROS). Excessive ROS can damage cellular proteins through oxidation, leading to misfolding and aggregation of proteins. Moreover, excessive intracellular ROS can damage genomic DNAs, leading to genome instability that will eventually create proteotoxic stress. For example, aneuploidy and gene amplifications can lead to imbalanced protein dosage and increased protein production. Point mutations and chromosome translocations can generate highly unstable, chaperone-dependent mutant proteins. In turn, these quantitative and qualitative changes in cancer proteome demand increased protein clearance, putting strain on the ubiquitin-proteasome system (UPS) of cancer cells. In order to maintain their robust fitness, cancer cells mobilize the powerful heat-shock response via HSF1 activation to counteract the internal proteotoxic stress. HSPs: heat-shock proteins. HSF1: heat-shock factor 1.