When Cancer Fights Back: Multiple Myeloma, Proteasome Inhibition, and the Heat-Shock Response
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Abstract

Multiple myeloma is a plasma cell malignancy with an estimated 26,850 new cases and 11,240 deaths in 2015 in the United States. Two main classes of agents are the mainstays of therapy—proteasome inhibitors (PI) and immunomodulatory drugs (IMiD). Other new targets are emerging rapidly, including monoclonal antibodies and histone deacetylase (HDAC) inhibitors. These therapeutic options have greatly improved overall survival, but currently only 15% to 20% of patients experience long-term progression-free survival or are cured. Therefore, improvement in treatment options is needed. One potential means of improving clinical options is to target resistance mechanisms for current agents. For example, eliminating the cytoprotective heat-shock response that protects myeloma cells from proteasome inhibition may enhance PI-based therapies. The transcription factor heat-shock factor 1 (HSF1) is the master regulator of the heat-shock response. HSF1 is vital in the proteotoxic stress response, and its activation is controlled by posttranslational modifications (PTM). This review details the mechanisms of HSF1 regulation and discusses leveraging that regulation to enhance PI activity. Mol Cancer Res; 13(8); 1163–73. ©2015 AACR.

Introduction

From 1971 to 1996, the overall survival rate for patients with multiple myeloma remained largely unchanged (1). Despite the use of alkylators, corticosteroids (dexamethasone and prednisone), and autologous bone marrow transplantation, little improvement was noted. Then, in 1999, thalidomide (in combination with dexamethasone) became the first new agent with major activity against multiple myeloma in 37 years (2). Thalidomide (Thalomid—FDA approval 2006) belongs to a class of structurally similar drugs known as immunomodulatory drugs (IMiD), along with lenalidomide (Revlimid—2006) and pomalidomide (Pomalyst—2013). IMiDs have helped to improve patient outcomes in recent years along with another major class of multiple myeloma agents: proteasome inhibitors (PI; ref. 3). The two FDA-approved PIs are bortezomib (Velcade—2003) and carfilzomib (Kyprolis—2012).

Proteasome Inhibition

The main effector in the ubiquitin–proteasome system (UPS) is the proteasome, a cytoplasmic protein complex responsible for protein degradation (4). The 26S proteasome is about 2,000 kilodaltons (kDa) in molecular mass and consists of one 20S protein subunit and two 19S regulatory cap subunits. Proteasomal degradation removes denatured, misfolded, damaged, or improperly translated proteins from cells. The UPS plays an essential homeostatic role in regulating intracellular protein concentration, as well as being a regulator involved in many cellular processes, including DNA repair, sodium channel function, regulation of immune and inflammatory responses, signal transduction, and cell-cycle progression (5). Proteasome-mediated degradation is particularly vital for plasma cell quality control because of its role as a professional secretory cell that produces copious amounts of immunoglobulin in a constitutive manner. Therefore, proteasome inhibition can dramatically alter protein homeostasis leading to stress responses and if not resolved, apoptosis (6).

Bortezomib is a highly selective and reversible PI that has a boron atom that binds the β5 subunit (PSMB5)/chymotrypsin-like activity of the 26S proteasome (7). The proteasome has an ATP-dependent proteolytic activity, therefore, bortezomib’s targeting of β5 results in decreasing or loss of proteasome function. Bortezomib was first reported as an anti-inflammatory agent for treating polyarthritis in 1998. Palomba and colleagues (8) used bortezomib as a means for inhibiting NF-κB activation by preventing proteasome-mediated degradation of IkBα, an NF-κB negative regulator. For cancer, bortezomib was first tested in vitro by Adams and colleagues (9), in a 60 tumor cell line NCI screen, and was most potent in the prostate cancer cell line, PC-3. Cytotoxicity was speculated to be due to stabilization and dysregulation of cyclins, CDK inhibitors, tumor-suppressor proteins, IkB, and other proteins associated with cell-cycle progression. Hideshima and colleagues (10) published the first report on bortezomib in multiple myeloma cell lines and freshly isolated patient samples. In addition to the NF-κB mechanism described above, bortezomib was shown to alter cellular interactions and...
cytokine secretion in the bone marrow milieu to inhibit tumor cell growth, induce apoptosis, and overcome drug resistance. Mit-stades and colleagues (11) used high-dose bortezomib in the human multiple myeloma cell line, MM.1S to probe gene expression changes. These changes included a downregulation of growth/survival signaling pathways, upregulation of molecules implicated in proapoptotic cascades, and upregulation of ubiquitin–proteasome pathway members and heat-shock proteins (HSP). HSP27, 40, and 70 upregulation was seen as early as 2 hours after treatment. Bortezomib was FDA-approved in 2003 for patient use in large part due to the results of a phase II study of its use in relapsed/refractory multiple myeloma (12).

Up to this point, while gene expression profiling had been used to characterize the molecular sequelae of bortezomib treatment, mechanisms mediating anti-multiple myeloma activity had not yet been defined. Questions remained unanswered, including “through what pathway(s) does PI induce apoptosis?” and “is there a cellular event specific to plasma cells that can predict its effectiveness?” Hideshima and colleagues (13) began to scratch the surface of the bortezomib-cell biology connection by linking bortezomib, p53 phosphorylation, JNK activation, caspase-3 and 8 activation, inhibition of DNA damage repair, and cell death. This study led to further investigation into the cell biology changes caused by bortezomib. However, what had not been looked at up to that point was specifically the plasma cell nature of a myeloma cell. Because of their role as immunoglobulin pro-ducers, plasma cells are heavily reliant on the unfolded protein response (UPR) for protein quality control (14). Lee and colleagues (15) suggested that UPR inhibition through IRE1α–UPR transmembrane suppression and splicing impairment of its downstream target, XBP1, plays a role in multiple myeloma PI-induced death. Our group showed that PIs can lead to an accumulation of misfolded proteins and an induction of terminal components of the UPR, including PERK, eIF-2α, ATF4, and its downstream target, CHOP (16). This was one of the first reports detailing how bortezomib was exploiting plasma cell biology, specifically immunoglobulin accumulation and terminal UPR activation, to induce apoptosis. Meister and colleagues (17) concluded that bortezomib-induced apoptosis is associated with the buildup of defective ribosomal products (DRIP) and other unfolded proteins in the endoplasmic reticulum (ER). Also, Bianchi and colleagues (18) determined that the balance between proteasome workload and degradative capacity represents a critical determinant of apoptotic sensitivity of multiple myeloma cell lines to PI. Fur-thermore, Ling and colleagues (19) have shown that low XBP1 levels predict poor response to bortezomib, both in vitro and in multiple myeloma patients, and ATF6 (a UPR transducer) expres-sion correlates with bortezomib sensitivity. Leung-Hagesteijn and colleagues (20) proposed that the existence of PI-insensitive Xbp1s+ tumor progenitors within primary multiple myeloma tumors may produce class-effect PI resistance independent of drug identity. Mechanistically, Xbp1s suppression induces borte-zomib resistance via decoupling of the ER to plasma cell maturation and immunoglobulin production, diminishing ER stress–asso-ciated cytotoxicity.

In addition to direct inhibition of the proteasome, PI-induced ER stress can also occur from aggresome formation and autophagy (21–23). Both are thought to be survival mechanisms used by cancer cells, and a recent study suggests that targeting the inte-grated networks of aggresome formation, proteasome, and autop-hagy may potentiate ER stress–mediated cell death pathways (21). However, one potential counter to PI effectiveness is the develop-ment of acquired mutations.

The direct target of bortezomib, PSMB5, is the most well-characterized mutation site (24). The PSMB5 mutation A49T has been shown to play a role in bortezomib resistance (25, 26). This mutation reduces bortezomib-induced apoptosis through the prevention of ubiquitinated protein accumulation and fatal ER stress in multiple myeloma. Despite this concern, no clinical evidence of an acquired proteasome subunit mutation has been published (25).

With the success of bortezomib in the clinic, second-generation PIs have been developed that have different activities, bioavail-ability (oral) and toxicity profiles. These agents have been the subject of intense preclinical and clinical studies. The first of these new inhibitors, carfilzomib, has now been FDA-approved for the treatment of relapsed/refractory multiple myeloma. Carfilzomib is an intravenous irreversible PI that binds to β5 with greater selectivity than bortezomib (27), NPI-0052 (marizomib), ONX 0912 (oprozomib), and MLN9708/2238 (ixazomib) are all in clinical trials (7, 27). Marizomib is being tested intravenously and oprozomib and ixazomib are being tested orally in multiple myeloma. Marizomib is a β-lactone–γ-lactam inhibitor that irreversibly binds β2 and β5 with high affinity and β1 with low affinity, and was granted “orphan drug” status by the FDA for multiple myeloma treatment. Phase I combination studies are being conducted using marizomib, pomalidomide, and dexa-methasone in subjects with relapsed/refractory multiple myelo-ma (28). Oprozomib is an epoxyketone that irreversibly binds β5 with high affinity and was also recently granted “orphan drug” status by the FDA for multiple myeloma and Waldenström macroglobulinemia treatment. Ixazomib is a boric acid analogue that reversibly binds β5 with high affinity and at higher concentra-tions is able to inhibit β1 and β2. Two recently published companion reports from phase I oral ixazomib studies in relapsed/refractory multiple myeloma patients showed that 15% to 18% of patients achieved partial response or better with 76% reaching a state of stable disease or better in one of the studies (29, 30).

Continued improvement in current treatments and clinical trials, including those for second-generation PIs, has led some researchers to state that prolonged disease-free survival and a cure for a majority of patients are on the horizon (31). Improved disease-free survival can only occur if we can identify and target cellular resistance mechanisms. Resistance mechanisms, includ-ing HSP upregulation as part of the heat-shock response (HSR), can limit PI effectiveness. Therefore, inhibiting the HSR is a therapeutic opportunity for improving PI efficacy.

The HSR and HSPs

As mentioned above, HSP family members were reported amongst genes that were highly upregulated by bortezomib (11). The HSR is part of a cell’s internal repair machinery and maintains homeostasis under stressful conditions, including infection, inflammation, exercise, exposure to toxins or pharma-cologic agents, starvation, or hypoxia (32). This response is carried out by HSPs, many of which act as chaperones assisting in protein folding and establishment of proper conformation while also preventing undesired protein aggregation. HSPs are categorized into five families: (i) HSP70 superfamily, (ii) DNAJ (HSP40) family, (iii) HSPB (small HSPs) family, (iv) HSP90/HSPC family,
Heat-Shock Factor 1

HSF1 is one of four proteins (HSF1–4) involved in stress response and development (54). It is the factor primarily responsible for HSP gene upregulation when myeloma cells are treated with bortezomib (55). HSF1 also drives a heat-shock–independent tumorigenesis program supporting oncogenic processes such as cell-cycle regulation, signaling, metabolism, adhesion, translation, and reprogramming of neighboring stromal cells to permit a malignant phenotype (56, 57). HSF2 has a minor role during the stress response (58). HSF1–HSF2 heterocomplexes form under conditions of cell stress, including proteasome inhibition, and reprogramming of neighboring stromal cells to permit a malignant phenotype (56, 57). HSF2 has a minor role during the stress response (58).

**Regulation of HSF1 by Posttranslational Modifications**

Because HSF1 is present in an inactive form, activation is mediated through posttranslational modifications (PTM; Fig. 1). These include phosphorylation, sumoylation, and acetylation, in addition to 14–3–3 binding. Table 1 lists kinases and associated phosphorylation sites that have been shown or speculated to be involved with HSF1 dissociation (from the inert cytoplasmic heterotetramer), trimerization, nuclear translocation, HSE binding, transactivation, and HSR attenuation (71).

Soubrier and colleagues (72) have shown that PKCδ activates HSF1 by S333 phosphorylation in the stress responsive regulatory domain, potentially leading to dissociation of the repressive cytoplasmic HSF1–HSP90 interaction. HSF1–S333A, a mutant HSF1 lacking S333 phosphorylation, associated with endogenous HSP90 to a greater extent than did HSF1–S333E, a mutant HSF1 with constitutively active S333 phosphorylation (phosphomimetic). In addition, S333E was twice as efficient at activating HSF1 than S333A.

To date, no published phosphorylation events have been specifically linked to positive regulation of HSF1 trimerization.
HSF1 PTMs and activation life cycle. A. HSF1 activating (green) and repressive (red) PTMs are shown above. The bottom left box displays a PTM abbreviation key. Amino acids—K, lysine; S, serine; T, threonine. AD, activation domain; C, c-terminus; DBD, DNA-binding domain; LZ, leucine zipper domain; N, n-terminus; RD, regulatory domain. B. The HSF1 activation and attenuation cycle with associated PTMs is shown above. HSF1 forms a constitutively inactive heterotetramer with HSP 40, 70, and 90. (Continued on the following page.)

Figure 1.
Kim and colleagues (73) have shown that nuclear translocation is regulated by PLK1-mediated phospho(p)Serine(S)419, but has no role in HSE binding or transactivation. Also, Murshid and colleagues (74) demonstrated that shRNA against PKAα blocked S320 phosphorylation, preventing HSF1 nuclear translocation in addition to disrupting other activation events discussed below.

HSE binding and transactivation are distinct activation steps but are regulated by several common phosphorylation events. pS320 is critical for hsp70.1 promoter HSE binding, transactivation, and reversal of HSF1 nuclear export (74). CKII-mediated pT142 phosphorylation is also vital in HSE binding and transactivation (75). Soncin and colleagues have shown that a T142A mutant inhibits HSE-binding ready nuclear HSF1 and ultimately, HSP70B gene transcription. In addition, Holberg and colleagues (76) observed that the molar ratio between CaMKII-mediated S320 and repressive PTM sites determines the magnitude of transactivation. However, pS230 is not needed for either stress-induced HSE-binding activity or the formation of nuclear stress bodies (the main site of accumulated HSF1, RNA Pol II, and other RNA-binding proteins in stressed cells).

Table 1. HSF1 kinases, their targets, and functional consequences

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Amino acid target</th>
<th>Functional consequences</th>
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<tbody>
<tr>
<td>AMPKκα</td>
<td>S21</td>
<td>Represses HSE binding and promotes HSF1 binding to HSP90</td>
</tr>
<tr>
<td>CAMKII</td>
<td>S230</td>
<td>Promotes transactivation</td>
</tr>
<tr>
<td>Casein kinase II</td>
<td>T142</td>
<td>Promotes HSE binding and transactivation</td>
</tr>
<tr>
<td>CDK1</td>
<td></td>
<td>Meiosis regulation</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>S307, S326, S363</td>
<td>Represses HSE binding and transactivation and is required for 14-3-3ε binding (S307); promotes transactivation (S326) or may inhibit MEK phosphorylation (S326); may repress HSE binding and transactivation (S363)</td>
</tr>
<tr>
<td>GSK3α</td>
<td>S303</td>
<td>Promotes transactivation</td>
</tr>
<tr>
<td>JNK</td>
<td>TAD, S307, S320, S363</td>
<td>Promotes nuclear localization, HSE binding, transactivation, and may reverse nuclear export (S320); may repress HSE binding and transactivation (S363)</td>
</tr>
<tr>
<td>MAPKAP-K2</td>
<td>S121</td>
<td>Represses HSE binding and promotes HSF1 binding to HSP90</td>
</tr>
<tr>
<td>MEK</td>
<td>S326</td>
<td>Promotes transactivation</td>
</tr>
<tr>
<td>mTOR</td>
<td>S326</td>
<td>Promotes transactivation</td>
</tr>
<tr>
<td>P38MAPK</td>
<td>S326</td>
<td>Promotes transactivation</td>
</tr>
<tr>
<td>PI3K</td>
<td>S320</td>
<td>Promotes nuclear localization, HSE binding, transactivation, and may reverse nuclear export</td>
</tr>
<tr>
<td>PKAα</td>
<td>S326</td>
<td>Promotes HSE binding and transactivation</td>
</tr>
<tr>
<td>PKCα, θ, ζ</td>
<td>S335, S363</td>
<td>Promotes HSF1 dissociation from HSP90 (S333 [PKCα only]); may repress HSE binding and transactivation (S363)</td>
</tr>
<tr>
<td>PLK1</td>
<td>S216, S419</td>
<td>Mitosis regulation (S216); promotes nuclear translocation (S419)</td>
</tr>
<tr>
<td>Rim15</td>
<td></td>
<td>Yeast only; promotes HSE binding when PKA activity is lowered by glucose deprivation</td>
</tr>
<tr>
<td>RSK2</td>
<td></td>
<td>Represses HSE binding</td>
</tr>
<tr>
<td>S112/MAPK7</td>
<td></td>
<td>Represses trimerization</td>
</tr>
<tr>
<td>Snf1</td>
<td></td>
<td>Yeast only; promotes HSE transactivation under conditions of glucose deprivation</td>
</tr>
<tr>
<td>Yak1</td>
<td></td>
<td>Yeast only; promotes HSE binding when PKA activity is lowered by glucose deprivation</td>
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K298 sumoylation and S363 phosphorylation are associated with transactivation repression. Furthermore, K80 and K118 acetylation destabilizes HSE binding. In addition, S303 and S307 phosphorylation are involved in 14-3-3ε binding to HSF1, which helps facilitate its nuclear export. (5) Upon export, HSF1 either returns to its cytoplasmic inactive state or is degraded. A, acetylation; P, phosphorylation; S, sumoylation.
Interestingly, Raychaudhuri and colleagues (87) have shown that K298 is acetylated during the stress response in addition to K286. Catalyzed by the acetyltransferase EP300, K298 and K208 stabilize and prevent degradation of the HSE-bound HSF1 trimer. EP300 maintains HSF1 stability in a phosphorylation-independent manner (87). Ten potentially phosphorylated serines were replaced with alanines, yet HSF1 remained acetylation competent. Notably, HSF1 acetylation kinetics do not match those of transactivation (88). Stabilizing acetylation is delayed upon onset of HSF1 transactivation and persists when HSF1 activity and DNA binding have attenuated.

PTMs also mediate negative regulation. HSF1 is maintained in an inactive heterotetramer by constitutive phosphorylation at S121, S303, S307, and potentially S363. Liu and Thiele (89) have shown that the linker region enclosing pS121 might be a negative regulator of the monomer to trimer transition. Wang and colleagues (90) identified MAPKAP-K2 (MKII) as the pS121-specific kinase and noted that pS121 promotes cytoplasmic HSP90 binding to HSF1 to help maintain its inactive state. Another negative regulatory event is ERK1/2-mediated S307 phosphorylation, which has been shown to be a priming event for GSK3β-mediated phosphorylation of S303 (91). pS303 prevents HSF1 trimerization upon stress-induced activation. Thus, the priming requirement by pS307 provides a potential link between the MAPK cascade and HSF1.

However, a contrasting study by Batista-Nascimento and colleagues (92) has shown that when human HSF1 was expressed in yeast, Slt2 (MAPK7) phosphorylated S303 independently of both GSK3β and the pS307 priming event. The authors concluded that differences in HSF1 structure between in vitro and in vivo systems may help to explain why different kinases can mediate S303 phosphorylation under different conditions. Downstream of these phosphorylation events, Wang and colleagues (93) have shown that both GSK3β-mediated pS303 and ERK1-mediated pS307 are prerequisites for HSF1–14-3-3ε binding. HSF1–14-3-3ε binding results in cytoplasmic HSF1 sequestration, specifically of the active, DNA-binding trimers. In addition, Chu and colleagues (94) demonstrated that pS363 is an early negative regulatory event that ultimately decreases HSF1β promoter activity though exactly where this phosphorylation event occurs is unclear. Contrasting studies suggest S363 is phosphorylated by PKCζ/δ (in vivo and in vitro), JNK (in vitro), or ERK (in vitro); ref. 91, 94, 95).

Postnuclear translocation negative regulation decreases HSF1 activity through a variety of mechanisms, ultimately leading to HSF1 release from the promoter region of its target gene(s) and export back to the cytoplasm. For example, pS121 can also inhibit HSE binding (90). In contrast to the positive regulation K298 sumoylation described above, Brunet Simionetti and colleagues (96) have published on a SUMO-2/3 modification at K298 that has been shown to block transactivation capacity. pS303 is also a prerequisite for this modification. Large HSP27 oligomers were shown to act as an E3 factor and serve as a scaffold to strengthen the repressive interaction between the SUMO-E2–conjugating enzyme, Ubc9, and HSF1. Furthermore, Raychaudhuri and colleagues (87) published on two destabilizing acetylation sites, K80 and K118. K80 and K118 acetylation occurs within the HSF1 DNA binding domain (amino acids 16–123) and these events lead to inhibition of chromatin binding by HSF1. This is a crucial step in the regulated release of HSF1 trimers from DNA, ultimately leading to HSR attenuation. K118 is positively regulated by EP300 like its stabilizing counterparts K208 and K298. (K80 was shown to be EP300-independent.) K118 is negatively regulated by the deacetylase, SIRT1. SIRT1 is regulated by AROS (a deacetylase promoter) and DBC1 (a deacetylase inhibitor). Raynes and colleagues (97) demonstrated that AROS and DBC1 have an impact on HSF1 acetylation status, HSF1 recruitment to the hsp70 promoter, and hsp70 transcription.

In addition to the roles described above, pS303 and pS307 have also been linked to accelerated HSF1 nuclear export through 14-3-3ε (93). 14-3-3ε binding influences HSF1 interaction with the nuclear export protein CRM1 and leads to enhanced nuclear export. 14-3-3β binding has also been linked to HSF1 nuclear export (98). Ultimately, a better understanding of positive and negative regulation through HSF1 PTMs may lead to treatments that alter HSF1 activation and help increase the efficacy of PI3K–based multiple myeloma therapy.

**HSF1 Inhibition in Cancer Treatment**

Targeting HSF1 could be a more effective therapeutic strategy than pursuing individual HSP inhibition. However, developing transcription factor inhibitors is difficult for many reasons. One, transcription factors bind negatively charged DNA and therefore their exposed regions are largely positive. This requires that any inhibitor must be negatively charged, but charged molecules cannot freely diffuse across the cell membrane. In addition, the DNA–protein interface is large and developing effective small-molecule inhibitors is difficult. To cover the entirety of their binding pockets, a large molecule may have to be developed. Bioavailability may become a concern and promiscuous binding to other targets could cause side effects. Finally, screens for transcription factor inhibitors are less straightforward than those for kinase inhibitors, which are reliant on easier to detect processes such as ATP hydrolysis or phosphate transfer to a substrate. Despite these complexities, multiple HSF1 inhibitor screens have been performed and their various methods are described below.

Whitesell and Lindquist (99) detailed drug-like inhibitors of the HSF1-regulated HSR and concluded that all HSP induction inhibitors suffer from low potency and/or poor specificity. At the time of that publication, those inhibitors included quercetin and its prodrug QC12, NZ28, and its structural analogue emunin, KNK437, strengerin B, and triptolide. Table 2 is an updated HSF1 inhibitor listing and Fig. 2 is an illustration of published inhibitor mechanisms. NZ28/emunin and triptolide will be discussed in detail below along with recently published inhibitors, cantharidin, 2,4-bis(4-hydroxybenzyl)phenol, KRBB11, and rohinitib.

NZ28/emunin was discovered as the result of a high-throughput screen for small molecules that inhibit HSP induction (100). The first step was performing a cell-based screen for inhibitors of

<table>
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<tr>
<th>Compound</th>
<th>Class</th>
<th>Positive HSF1 inhibition</th>
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<tr>
<td>2,4-bis(4-hydroxybenzyl)phenol</td>
<td>Terpenoid</td>
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<tr>
<td>Cantharidin</td>
<td>Emetine derivative</td>
<td></td>
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<tr>
<td>Emunin</td>
<td>Emetine derivative</td>
<td></td>
</tr>
<tr>
<td>KNK437</td>
<td>Benzylidene lactam</td>
<td></td>
</tr>
<tr>
<td>KRBB11</td>
<td>Diaminopyrimidine</td>
<td></td>
</tr>
<tr>
<td>NZ28</td>
<td>Emetine derivative</td>
<td></td>
</tr>
<tr>
<td>QC12</td>
<td>Quercetin produg</td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>Flavonoid</td>
<td></td>
</tr>
<tr>
<td>Rohinitib</td>
<td>Flavagline derivative</td>
<td></td>
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<tr>
<td>Strengerin B</td>
<td>Streptomyces fermentation product</td>
<td></td>
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<tr>
<td>Triptolide</td>
<td>Diterpene triepoxide</td>
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HSP-mediated refolding of heat-denatured luciferase followed by a counterscreen for toxicity. The second step was direct testing for HSP induction inhibition by immunoblotting against HSPA70. Out of 20,000 compounds from several diversity libraries, emunin was found to sensitize PC-3 human prostate cancer cells and MM1.S to proteasome and HSP90 inhibitors without significant toxicity. However, its precise mechanism of HSP translation inhibition mechanism is unknown, and may involve events downstream of HSF1, leading to significant concerns over specificity (99).

Triptolide is a diterpenoid epoxide derived from *Tripterygium wilfordii*, a plant long used in Chinese medicine (101). Heimbberger and colleagues (102) used triptolide to take advantage of a myeloma cell’s sensitivity to proteasome inhibition and subsequent reliance on the cytoprotective HSR. In MM.1.S and INA-6 myeloma cell lines, triptolide in combination with bortezomib synergistically induced apoptosis. Although this is a promising result, concerns about the specificity of this agent exist. Triptolide interferes with NF-κB, NFAT, AP-1, and p53 activity, and inhibits global gene transcription by inducing RNA Pol II degradation and inhibiting the ATPas activity of the DNA helicase ERCC3 (103). In addition, the in vivo tumor model in mice measuring tumor burden did not extend past 11 days, which raises the question about the durability of triptolide’s in vivo effects (102). Although triptolide holds promise as a multiple myeloma therapeutic, its specific mechanisms must be better understood.

Yoon and colleagues (104) identified KRIBB11 from a synthetic chemical library screen. A heat-shock-dependent luciferase reporter plasmid was used to identify HSF1 inhibitors and KRIBB11 was chosen for further testing from an approximately 6,230 compound chemical bank. KRIBB11 abolished heat-shock-dependent HSP70 induction through HSF1 inhibition in colon carcinoma HCT-116 cells and also inhibited the growth of HCT-116 cells in a nude mouse xenograft regression model. KRIBB11 inhibited P1 or HSP90 inhibitor-mediated HSP induction, indicating its potential use in combination therapy. Interestingly, while KRIBB11 does not inhibit heat-shock–induced recruitment of HSF1 to the hsp70 promoter, it does inhibit P-TEFb (positive transcription elongation factor, a heterodimer of CDK9 and cyclin T) recruitment. This study was able to show by affinity chromatography and competition assays that KRIBB11 specifically inhibits HSF1. In the competition assay, HSF2, HSP90, and CDK9, common HSF1 binding partners, were not detected, thus further strengthening the argument that KRIBB11 is HSF1 specific. In a separate study, Wiita and colleagues (105) combined KRIBB11 with low-dose bortezomib in MM.1.S and saw an additive apoptotic effect. KRIBB11 shows HSF1 specificity and will be worth monitoring as it progresses through further preclinical studies.

Kim and colleagues (106) identified the blister beetle–derived compound cantharin as an HSF1 inhibitor from a similar screen to the one used for KRIBB11. Cantharin was shown to have inhibitory effects on HSP70 and BAG3 expression in HCT-116 cells. Here, cantharin blocked HSF1–dependent P-TEFb recruitment to the HSP70 promoter. Cantharin demonstrated anti-cancer effects and an additive effect with bortezomib, but its HSF1 specificity is questionable. Cantharin is known as a P2PA inhibitor (107). In addition, it has also been shown to be an activator of serine proteases in epidermal cells (108).

Another natural compound, 2,4-bis(4-hydroxybenzyl)phenol derived from the orchid *Castotia elata*, was identified from a screen using a luciferase reporter under the control of a HSE to find inhibitors of HSF1 activity in NCI-H460 human lung cancer cells (109). Similar to the previously mentioned studies, data from Yoon and colleagues indicate that (1) can lead to HSP suppression and an increase in apoptosis. The mechanism proposed is that (1) induces degradation of HSF1 through S326 dephosphorylation. However, HSF1 knockdown with siHSF1 + (1) resulted in increased degradation compared to (1) alone, yet cell death with siHSF1 + (1) is less than that of (1) by itself. Therefore, while this study points to a specific mechanism by which its compound works, more work is needed to confirm that observation.

Santagata and colleagues (110) used a 300,000+ compound chemical screen to look for HSF1 inhibitors and found that the rocагламиде, rocагламиде A, was the most potent and selective hit. Rocагламиде A inhibits translation initiation factor eIF4A, thus providing a link between HSF1 and protein translation flux. Rocaglate specificity for HSE reporter activity inhibition was demonstrated by stably transducing NIH3T3-HGL mouse embryonic fibroblasts with two constructs; one encoding a green fluorescent protein (GFP) driven by HSEs and the other encoding a red fluorescent protein (RFP) driven by a doxycycline-regulated control promoter. Rocaglates suppressed GFP but not RFP activity, whereas triptolide, quercetin, and KKNK437 (among other previously reported HSF1 inhibitors), suppressed both GFP and RFP. An analogue, rohinitib (for Rocaglate Heat Shock), was found to be more potent than rocагламиде A while retaining similar selectivity and was used for in vivo mouse studies. An M0-91 mouse myeloma cell xenograft (AML) xenograft model showed that rohinitib treatment resulted in significantly decreased tumor volume in addition to a dramatic reduction in HSPA8 mRNA. However, rocагламиде derivatives are known to inhibit NF-κB and therefore, rohinitib HSF1 specificity needs to be looked at in further detail (111). Regardless, investigation of the relationship between the ribosome, translation flux, and HSF1 will provide novel insight into targeting the biology of a cancer cell.

As noted earlier, the main difficulty of finding small-molecule transcription factor inhibitors stems from the size and complexity of the DNA–protein interface. In this regard, RNA aptamer technology may prove useful. RNA aptamers are small oligonucleotides that specifically bind to targets such as small proteins (112). RNA molecules share some common structural features with DNA, and RNA aptamers have been shown to target the DNA-binding domains of molecules such as NF-κB. Although aptamer technology is in its infancy as a therapeutic strategy, it can currently be used for drug target validation. For example, Salamanca and colleagues (113), modified iRNAHSF1, a *Drosophila* RNA aptamer, to block HSE binding in HeLa cells and promote apoptosis.

In addition to direct HSF1 inhibition, targeting its activation by modulating PTMs is also a potential therapeutic strategy. HSF1 PTMs happen in all stages of activation and attenuation as previously described. The majority of published studies on HSF1 PTMs focus on phosphorylation events and their respective kinases. For example, the aforementioned study describing how HSP90 inhibition leads to nuclear HSF1 accumulation also showed that that accumulation was reduced by mTOR inhibition (53). Therefore, targeting kinases that activate HSF1 could be a simpler way of modulating targeting this pathway than developing HSF1 inhibitors.
Taken together, the findings described here show that HSF1 is involved in several cancers, including multiple myeloma. HSF1 has drawn interest as a biomarker though there are no known translocation groups or mutations associated with its activity (57, 114, 115). A broad variety of tumors, including carcinomas of the breast, cervix, colon, lung, pancreas, and prostate, as well as mesenchymal tumors such as meningioma, show increased HSF1 gene copy number, protein expression, or activation compared to their normal counterparts (56, 116). Dai and colleagues (117, 118) have shown a therapeutic window between cancer and normal cells by demonstrating that HSF1 depletion minimally affects normal cell viability, whereas cancer cells are strongly affected by HSF1 depletion. HSF1 inhibitors will likely play a role in treating a diverse range of malignancies, including multiple myeloma, because of HSF1’s multifaceted role in promoting tumorigenesis (56, 57). We anticipate that one target multiple myeloma population will be those who are bortezomib-resistant. An HSF1 inhibitor could help unblock one potential bortezomib resistance mechanism and increase multiple myeloma apoptosis.

Although there is a demonstrated need for an HSF1 inhibitor, the future of HSF1 drug development will depend, in part, on the ability for therapeutic agents to be able to effectively and specifically target HSF1. Direct HSF1 inhibition has proven to be an elusive task, but the studies presented demonstrate progress. In addition to direct inhibition, new drugs could target HSF1 activation through PTM inhibition; for example, kinase or histone deacetylase (HDAC) inhibition or anti-SUMO therapies. Regardless of the mechanism, drugs should show the ability to work in tandem with current therapies, such as proteasome inhibition, because the majority of current induction therapy is based on combination and not single-agent treatments.

**Conclusions**

There is no universal cure for multiple myeloma, but recently developed therapies such as IMiDs and PIs have dramatically increased patient survival. Bortezomib is effective in multiple myeloma therapy for a variety of reasons, including targeting its plasma cell biology. However, multiple myeloma cells counter bortezomib treatment by activating the HSR. This cytoprotective mechanism is regulated by the master transcription factor, HSF1. Developing a specific and effective HSF1 inhibitor has proven to be a challenge. While that aim is being pursued, a more practical approach is targeting HSF1 regulation. This strategy could have a dramatic impact on patient survival, especially when combined with current PI-based therapies, even beyond multiple myeloma. A genome-wide siRNA screen identified proteasome addiction as a vulnerability of basal-like triple-negative breast cancer (TNBC) cells (119). Multiple
myeloma, TNBC, and bladder cancer are three examples of malignancies whose patients could benefit from a therapeutic strategy of proteasome and HSF1 inhibition.

Disclosure of Potential Conflicts of Interest
S. Lonial is a consultant/advisory board member for Millennium, The Takeda Oncology Company, Celgene, Novartis, Bristol-Myers Squibb, Onyx Pharmaceuticals, Janssen Pharmaceutical Companies, The Pharmaceutical Companies of Johnson & Johnson. L.H. Boise is a consultant/advisory board member for Onyx Pharmaceuticals and Novartis. No potential conflicts of interest was disclosed by the other author.

References


59. Pockley AG, Calderwood SK, Santoro MG. Prokaryotic and eukaryotic heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) is an enabler of malignancy. Cell 2014;158:564–78.


