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Regulating the master regulator: Controlling heat shock factor 1 as a chemotherapy approach

Jeanette R. McConnell, Laura K. Buckton, Shelli R. McAlpine *

Department of Chemistry, University of New South Wales, Sydney, NSW 2052, Australia

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ABSTRACT

Described is the role that heat shock factor 1 (HSF1) plays in regulating cellular stress. Focusing on the current state of the HSF1 field in chemotherapeutics we outline the cytoprotective role of HSF1 in the cell. Summarizing the mechanism by which HSF1 regulates the unfolded proteins that are generated under stress conditions provides the background on why HSF1, the master regulator, is such an important protein in cancer cell growth. Summarizing siRNA knockdown results and current inhibitors provides a comprehensive evaluation on HSF1 and its current state. One set of molecules stands out, in that they completely obliterate the levels of HSF1, while simultaneously inhibiting heat shock protein 90 (Hsp90). These molecules are extremely promising as chemotherapeutic agents and as tools that may ultimately provide the connection between Hsp90 inhibition and HSF1 protein levels.

The survival transcription factor heat shock factor 1 (HSF1) plays a critical role in rescuing the cell when it is under stress. Specifically, HSF1 is vital for promoting the refolding and deaggregation of proteins required to maintain the cell's survival (Fig. 1a). This role of HSF1 in promoting protein folding becomes even more important for malignant cells.

HSF1 is overexpressed in numerous cancer types, including: breast, prostate, kidney, colon and nerve sheath and this overexpression is correlated with increased malignancy and mortality.[1–3] For example, high levels of HSF1 are present in the metastatic breast cancer cell line BPLER, where as low levels of HSF1 are present in its less metastatic counterpart HMLER.[4] The negative impact of high HSF1 levels also translates into patient samples. Indeed, 48% of high-grade cancers had high levels of HSF1 while only 14% of the low-grade carcinomas had high HSF1 levels.[1,4] Patient samples of hepatocellular carcinoma showed a 4.6-fold increase in HSF1 mRNA compared to normal tissues, and in multiple myeloma, seven out of eight metastatic patient samples examined were HSF1 positive.[5] These elevated levels of HSF1 correlate with patient outcome.[5] High cellular levels of HSF1 indicated a poor prognosis in hepatocellular carcinoma and the same was true for high nuclear HSF1 levels in breast cancer patients.[1] Elevated HSF1 levels clearly support malignancy and thus demonstrate that HSF1 is important in maintaining the highly malignant phenotype, and progression of cancer.

HSF1's primary role is to protect a cell when it is under stress. Thus, HSF1 is highly important in cancer, because cancer cells are constantly in a state of stress. One reason for this stressed state is that the cancer cells are rapidly dividing and require the quick generation of large numbers of proteins.[6] When these proteins are not folded fast enough unfolded proteins accumulate, which triggers a 'heat shock response' (HSR).[7] A HSR is depicted in Figure 2 and summarized below.

When large amounts of unfolded protein begin to accumulate, HSF1 is released from its repressive complex with Heat shock protein 90 (Hsp90).[2,8,9] The release of HSF1 leads to its activation, beginning with the formation of an HSF1 trimer complex (Fig. 2).[10] The trimer becomes phosphorylated at more than 10 sites, many of which are between amino acids 292–363 in the HSF1 protein, while phosphorylation of Ser326 is the most important for activity.[10,11] Multiple kinases are responsible for the phosphorylation of HSF1 and these kinases are the targets of several anti-HSF1 drugs.[2] Once HSF1 is trimerised and phosphorylated it translocates to the nucleus where it fulfils its duty as a transcription factor and binds to the DNA elements that initiate the HSR.[10] This leads to a quick and massive up-regulation of inducible heat shock proteins (HSPs), specifically Hsp90, 70, 40, 27, and activation of HSF1.[7,10]

The upregulation of the HSPs is an important part of HSF1's cancer supporting mechanism, but it is not the only reason cancer cells

* Corresponding author. Tel.: +61 4 1672 8896; fax: +61 2 9385 6141.
E-mail address: s.mcalpine@unsw.edu.au (S.R. McAlpine).

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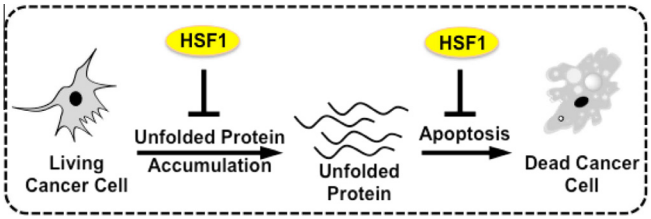

Figure 1. HSF1 blocks apoptosis caused by unfolded proteins. HSF1 prevents the toxic build up of unfolded proteins by facilitating protein folding. Reducing the amount of unfolded proteins ultimately protects the cell from the apoptosis that would otherwise ensue.

rely on HSF1. The role of Hsp90, 70, 40, and 27 in cancer cell growth is reasonably well described in the recent literature.[12–21] However, it is often overlooked that HSF1 contributes to the malignant phenotype though mechanisms distinct from the HSR. In cancer cells more than 60% of the DNA elements bound by HSF1 are unique from those driven by heat shock.[4] This indicates that in addition to inducing the heat shock response and driving resistance through elevated heat shock protein levels, HSF1 promotes other factors, which contribute to cancer. The majority of these other proteins induced by HSF1 are involved in translation, metabolism, and cell adhesion; each of which play important roles in the development and progression of cancer.[4] This additional support that HSF1 provides to cancer growth indicates it is an outstanding oncogenic target, where inhibiting HSF1 should lead to shutting down not only the HSR, but also other highly relevant pathways supported by HSF1.

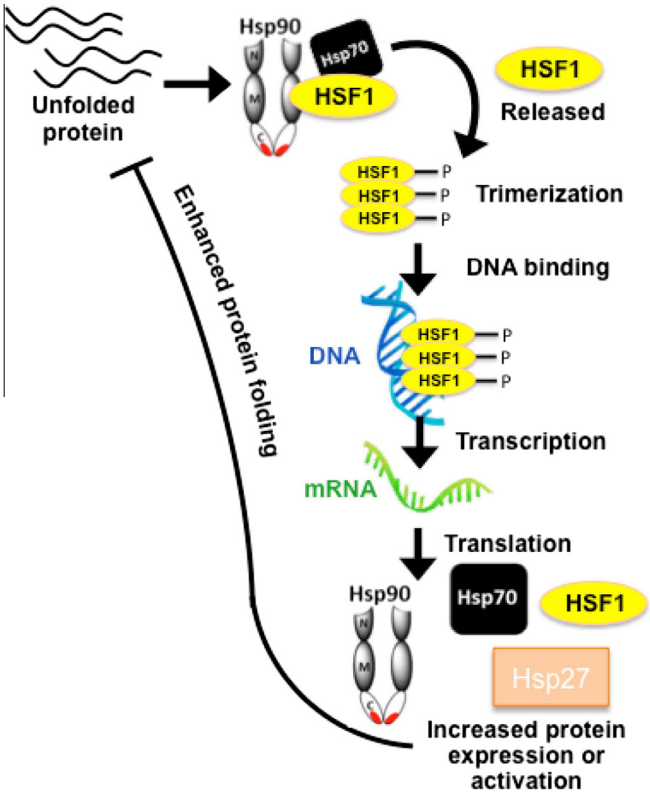

Figure 2. The heat shock response pathway. Accumulation of unfolded proteins initiates the release and subsequent trimerization and hyperphosphorylation of HSF1. HSF1 then binds DNA and initiates upregulation of HSP mRNA and protein, including Hsp27 and Hsp70. The extra HSPs assist in protein folding, lessening the burden of unfolded proteins on the cell.

The investigation of HSF1 knockdown highlighted the importance of this protein in cancer growth and initiation. The knockdown of HSF1 via siRNA or genetic mutation greatly diminishes tumorigenicity in a variety of cancer types. In the triple negative breast cancer cell line, Hs578T, treatment with siRNA against HSF1 reduced the cell survival rate by 40%.[22] In vivo HSF1 knockdown prevented 8/11 mice injected with HER2+ MMTVneu cells from forming tumours, while all HSF1+ mice formed tumours.[23] In addition, mice with HSF1 knocked down and then injected with HER2-expressing breast cancer cell line MCF-10A also failed to form tumours until HSF1 expression had returned.[24]

HSF1 was also necessary for the formation of RAS induced tumors.[25] Knock down of HSF1 reduced the formation of RAS melanoma tumors and increased the survival of mice bearing HSF1 negative RAS induced tumors by ~200 days compared to HSF1+ tumors.[2,25] A similar effect was seen in the p53 mutant induced tumors, where HSF1 knock down halted the formation of tumors caused by the loss of p53 function.[2] This comprehensive in vivo evidence of the explicit necessity of HSF1 for tumour transformation in three different cancer models demonstrates that HSF1 is a promising anti-cancer target, and also illustrates that its role in cancer development is much more important than was previously thought.

The overwhelming ability of HSF1 to support cancer growth brings into question the use of the 'classic Hsp90 inhibitors' of which there are currently 8 in clinical trials. These amino (N) terminal Hsp90 inhibitors have been used as anticancer drugs because Hsp90 is up-regulated in cancer cells and helps maintain the malignant phenotype by folding and activating many oncogenic proteins.[26] Inhibiting Hsp90 can kill cancer cells, but Hsp90 inhibitors have been most effective when used to sensitize cancer cells to other types of chemotherapeutics.[13,27] However, these drugs are known to induce the HSR, as well as cause increased HSF1 protein levels (Fig. 3a).[12–16,28,54] The induction of the HSR and elevated HSF1 promotes resistance. The classic Hsp90 inhibitors has been known to cause the HSR and increased HSF1 levels for the last 2 decades, however, the recent developments in the understanding of HSF1 and its integral part in cancer initiation highlights the need for other types of Hsp90 inhibitors.

Indeed, the most current data on Hsp90 inhibitors has now clearly shown that Hsp90 can be inhibited without increasing HSF1 levels.[12–15,17,28] Specifically, the SM series of compounds, whose most effective molecule, SM258, completely depletes the HSF1 protein in drug-treated cells via allosteric modulation of Hsp90's C-terminus (Fig. 3b).[28] In addition, a new Hsp90 inhibitor, which was recently reported directly blocks access to the C-terminus (Fig. 3, tetratricopeptide repeat (TPR) mimics).[29] Although this molecule (5.1 CYC) has not been tested for its ability decrease HSF1 levels, it has a similar mechanism to the SM series compounds and thus may also deplete the HSF1 protein.

Despite the negative side effect of the upregulation of HSF1 by the 'classic Hsp90 inhibitors' these drugs provide a great opportunity to test anti-HSF1 therapies in an environment that has become highly dependent on HSF1 (Table 1). For example, HSF1 knock down in A375 and A2058 melanoma cell lines sensitized these cells to the Hsp90 inhibitor NVP-HSP990, reducing the IC_{50} value from 19 nM and 12.7 nM to 6 nM and 5.2 nM, respectively.[30] Also, depletion of HSF1 in PC-3 prostate cancer cells increased the apoptosis caused by two other Hsp90 inhibitors 17-AAG and radicicol by 20% (in PC-3 cells) and 40% (in HCT-116 cells) respectively, compared to the Hsp90 inhibitor alone (Table 1).[31] This was true across other cell lines, where HSF1 knock down caused ~5-fold sensitization to 17-AAG in DU-145 and HCT-116 cells (Table 1).[32] Biological knockdown of HSF1 sensitized cells to the 'classic Hsp90 inhibitors' by abrogating the induction of the heat shock response. Clearly, HSF1 is having a very detrimental effect on the efficacy of 'classic