

Metabolic stressors disrupt proteome homeostasis to suppress malignancy

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Within tumor cells the heat shock factor 1 (HSF1)-mediated stress response is constitutively mobilized to counter persistent proteotoxic stress, thus sustaining their fragile proteome homeostasis and supporting robust malignant phenotypes. Intriguingly, our new studies reveal that metabolic stressors, such as metformin, inactivate HSF1 and provoke proteomic chaos, thereby impeding tumorigenesis.

Despite their aggressive behaviors, malignant cells constantly endure genotoxic, oxidative, and metabolic stress.¹ In response, a diverse range of cellular responses are mobilized to avert deleterious consequences inflicted by these distresses. Ironically, malignant cells hijack these defensive mechanisms for their own survival and prosperity.

Emerging evidence reveals that malignant cells also suffer proteotoxic stress,^{2,3} a type of distress that arises from perturbation of cellular proteome homeostasis, or proteostasis.⁴ Perturbagens could be either environmental or intrinsic, the latter of which are often causally related to human neurodegenerative disorders. In essence, proteostasis is the outcome of finely balanced production and disposal of cellular proteins. This delicate equilibrium is constantly challenged by a wide array of insults or stressors, many of which affect protein folding. It is widely recognized that proper folding is vital for both normal function and stability of proteins. Misfolded and aggregated proteins become detrimental and have to be cleared promptly by either the ubiquitin-proteasome or lysosome system. Malignant transformation inevitably increases protein synthesis, augments protein

oxidative damage, and produces proteins with altered conformations as a result of genetic mutations.^{2,3} Therefore, malignant transformation, like a stressor, disturbs cellular proteostasis in a cell-autonomous fashion.

To counter proteotoxic stress, cells have evolved a transcriptional program that is hallmarked by drastic induction of heat-shock protein (HSP) genes. This cytoprotective mechanism is named the heat-shock response, or proteotoxic stress response (PSR).³ As molecular chaperones, HSPs aid in the folding, transportation, assembly, and degradation of other proteins.³ Through regulation of HSPs, the PSR plays a critical role in guarding the proteome against misfolding and aggregation, thereby preserving proteostasis. In mammals, heat shock factor 1 (HSF1) is the master regulator of this powerful stress response.⁵ Not surprisingly, the HSF1-mediated PSR enhances cellular and organismal survival under stressful conditions and antagonizes diseases related to protein misfolding.³

Paradoxically, recent work from our group and other laboratories has uncovered a key pro-oncogenic role of this otherwise beneficial adaptive mechanism. In a broad range of human cancers HSF1

protein expression is elevated and positively correlated with malignant progression.^{6,7} Moreover, our studies have demonstrated constitutive activation of HSF1 in malignant cells even in the absence of environmental insults,^{6,8} highlighting intrinsically persistent proteotoxic stress. *Hsf1* deficiency impedes tumorigenesis in diverse mouse models^{2,6,9} and *HSF1* depletion markedly impairs the growth and survival of diverse human tumor cell lines.^{2,6} These results collectively pinpoint critical roles of HSF1 and the PSR in both tumor initiation and maintenance. Nevertheless, the precise molecular mechanisms underlying cell-autonomous regulation of HSF1 in malignancy remain largely unclear.

In light of its pro-oncogenic function, the PSR is logically considered a potential therapeutic target. In a search for small molecules that suppress constitutive activation of HSF1 in tumor cells, we identified metformin,⁸ the most commonly prescribed drug for patients with type 2 diabetes (T2D) worldwide. Intriguingly, epidemiological studies have revealed a reduction in overall cancer incidence in T2D patients taking metformin,¹⁰ suggesting a promising antineoplastic effect of metformin. Through blockade of the mitochondrial

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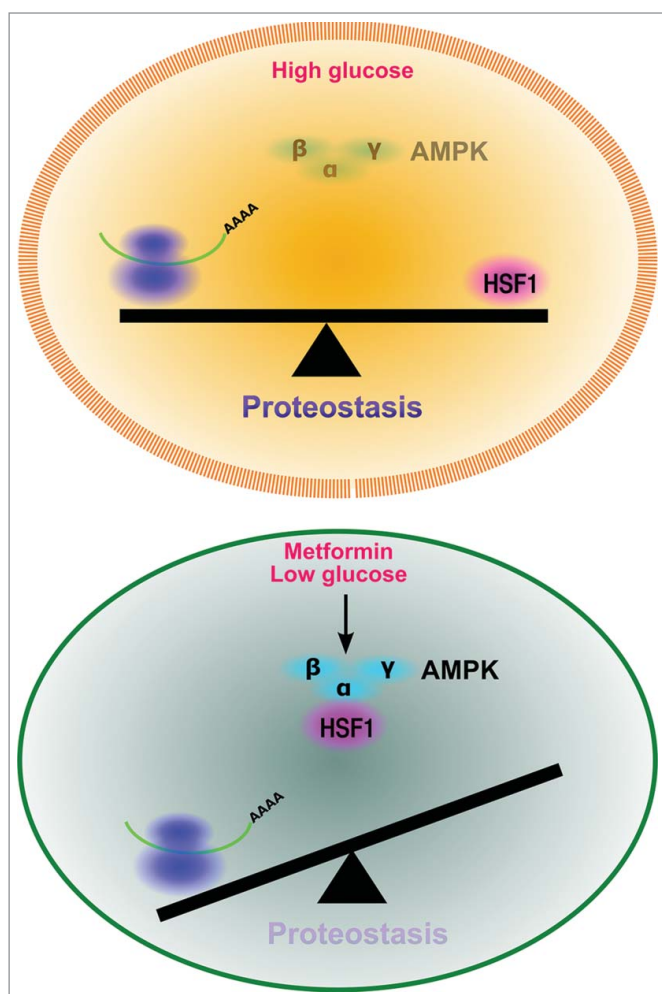


Figure 1. Metabolic stressors perturb proteostasis to suppress malignancy. Under nutrient-rich conditions, adenosine monophosphate (AMP)-activated protein kinase (AMPK, comprising α , β , and γ subunits) remains repressed and heat shock factor 1 (HSF1) is constitutively mobilized to supply sufficient cellular chaperoning capacity. Thus, the HSF1-mediated proteotoxic stress response (PSR) ensures the quality of the tumor cell proteome, thus preserving tumor proteostasis and supporting malignancy (top panel). In contrast, metabolic stressors such as metformin and nutrient deprivation mobilize AMPK to inactivate HSF1. In consequence, the fragile tumor proteostasis is disrupted and proteomic chaos ensues, leading to suppression of malignancy (bottom panel).

respiratory chain, metformin depletes cellular adenosine triphosphate (ATP) and thereby provokes metabolic stress.¹⁰ As a consequence, the systemic metabolic stress response (MSR) is elicited. The adenosine monophosphate (AMP)-activated protein kinase (AMPK) plays a central role in sensing fluctuations in cellular AMP/ATP ratio and mediating the MSR.¹⁰ Through a reduction in ATP expenditure and increase in ATP production, the MSR enables cells to restore energy homeostasis and survive metabolic stress.¹⁰

We found that metformin suppresses HSF1 activation through AMPK.⁸

Upon activation by metformin, AMPK physically interacts with and phosphorylates HSF1 at serine 121 (Ser121). Importantly, this modification not only impairs the transcriptional activities of HSF1, at least in part by impeding its nuclear translocation, but is also necessary for the HSF1 protein destabilization induced by metformin. Interestingly, like metformin, deprivation of leucine or glucose also suppresses the PSR via AMPK–HSF1 interaction. Accordingly, metabolic stress antagonizes the PSR, rendering cells vulnerable to proteotoxic stress.⁸

Beyond blocking the PSR induced by environmental insults, both metformin and glucose deprivation suppress constitutive activation of HSF1, and provoke global protein ubiquitination and aggregation in human tumor cell lines.⁸ In stark contrast, these metabolic stressors have no obvious effect on non-transformed cells. These results support the notion that malignant cells endure higher levels of proteotoxic stress than their non-transformed counterparts. Congruent with a causative role of HSF1 inactivation, expression of HSF1^{S121A} mutants antagonizes both the proteotoxic stress and inhibition of proliferation engendered by metformin in human melanoma cells.⁸ In support of its antineoplastic effects, a clinically relevant concentration of metformin retards *in vivo* melanoma growth. Interestingly, metformin not only induces HSF1 Ser121 phosphorylation but also depletes HSF1 proteins in melanoma cells.⁸ Not surprisingly, global protein ubiquitination and apoptosis are provoked in metformin-treated melanomas.⁸ Importantly, HSF1 overexpression markedly antagonizes the effect of metformin on proteostasis, survival, and growth in melanoma.⁸ Together, these findings strongly indicate that HSF1 inactivation contributes to the antineoplastic activity of metformin.

A key activity of HSF1 in malignancy appears to be to suppress proteotoxic stress and preserve a delicate proteostasis (Fig. 1). Unlike non-transformed cells, malignant cells rely on HSF1 for their fitness. It appears that proteomic stability empowers a robust oncogenic process and the malignant state is inherently associated with a heightened susceptibility to proteomic perturbation. Thus, disrupting the fragile proteostasis of cancer may represent an attractive therapeutic strategy. In principle, this can be achieved through debilitation of the HSF1-mediated defense, imposition of additional stress, or the combination of both.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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