COMMENTARY

Shining a light on early stress responses and late-onset disease vulnerability

Mark F. Mehler[a,b,c,d,1]

There is increasing evidence that neurodegenerative and neuropsychiatric disorders have developmental components to pathogenesis that encompass a prodromal phase associated with regional impairments in neural cell maturation and maintenance, neural network connectivity, and oscillatory synchrony (1, 2). These abnormal developmental states elicit a spectrum of homeostatic mechanisms and predispose to late-onset disease. Understanding the mechanisms governing cellular vulnerability to disease is essential for defining pathogenesis and for identifying selective biomarkers and earlier and more efficacious disease-modifying therapies. Moreover, these disorders are associated with comorbidities that may reflect the confluence of brain- and body-wide foci of cellular vulnerability involving specialized cell types, their associated niches and microenvironments, as well as interorgan communications (2–4). These disease-associated developmental processes are associated with various stressor states that promote relaxation of lineage constraints and aberrant reprogramming of cell fate (5). These observations may explain the findings of epidemiological studies that specific neuropsychiatric diseases exhibit cross-protective as well as mutually deleterious effects with systemic cancer subtypes that might have wider applicability to other diseases of aging (3). Genome-wide/epigenome-wide association studies have revealed that the majority of genomic variations in such age-related diseases occur in noncoding genome regulatory regions (6), which include enhancers, superenhancers, promoters, and insulators normally mediating transcriptional and epigenetic programs associated with cell fate, lineage plasticity, homeostasis, dynamic genome remodeling, and stress responses. However, the ability to better understand these intriguing pathogenic mechanisms has been hampered by an inability to properly assess the molecular, cellular, and system-wide consequences of the evolution of a spectrum of stressor states that predispose to various disease vulnerabilities and pathological outcomes.

In PNAS, Torii et al. (7) have developed molecular reagents and transgenic mice to detect, follow, isolate, and interrogate vulnerable cells in brain and body initially damaged in utero or during postnatal life by a broad but finite range of environmentally mediated stressor states, associated with threshold activation of heat shock factor 1 (HSF1) signaling pathways, and frequently linked to late-onset neuropsychiatric disorders. Torii et al. used a heat shock element (HSE)-RFP reporter system and generation of HSE-RFP transgenic mice for detection of cellular HSF1 activation. In addition, to control the period of HSE-dependent detection of the period of endogenous Hsf1 activation following stress induction and long-term tracing of surviving but vulnerable cells, Torii et al. used a plasmid containing a HSE–codon-optimized Flippase (FLPo) cassette flanked by LoxP sites transferred into a FRT-GFP/CAG-Cre-ER transgenic mouse. This process allows FLPo to be expressed after stress exposure, which induces GFP expression via FRT recombination, with subsequent activation of Cre recombinase following tamoxifen administration, leading to excision of the floxed HSE-FLPo cassette and tracing of sustained GFP expression independent of additional stress induction. In vitro and in vivo studies demonstrated the specificity, sensitivity, and critical period of the environmental challenges required to elicit endogenous Hsf1 expression and long-term tracing of descendant cells. Moreover, labeled cells exhibited early oxidative stress responses, preferential cerebral cortical neural progenitor cell targeting, alterations in diverse morphological and cell migratory parameters, and additional labeling of cells from multiple tissues and organs following systemic stress induction. The Torii et al. study follows previous pioneering observations of the authors that exposure of embryos to stressors and activation of endogenous Hsf1 in murine cerebral cortical cells increases susceptibility to late-onset neuropsychiatric disease phenotypes.

The work by Torii et al. (7) is intriguing and provocative because it will facilitate detailed cell type-specific subcellular, tissue/organ-relevant, systemic, and meta-omics examinations of previously unapproachable latent and vulnerable cells during the entire prodromal and manifest stages of disease in response

[a]Institute for Brain Disorders and Neural Regeneration, Albert Einstein College of Medicine, Bronx, NY 10461; [b]Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461; [c]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461; and [d]Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY 10461

Author contributions: M.F.M. wrote the paper.

The author declares no conflict of interest.

See companion article on page 2367.

[1]Email: mark.mehler@einstein.yu.edu.

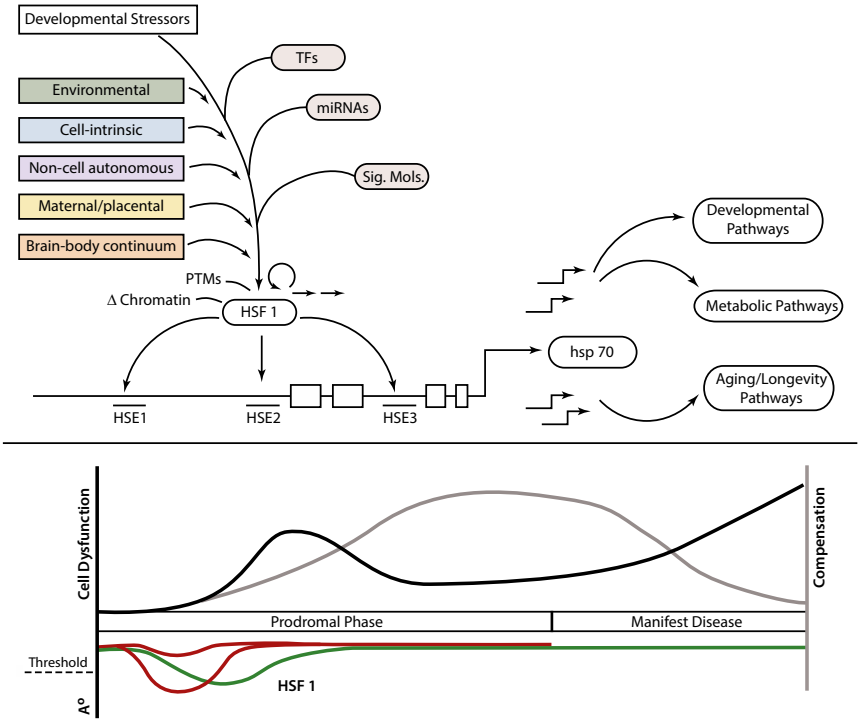

Fig. 1. Roles of HSF1 in developmental stress responses leading to late-onset disease states. (*Upper*) HSF1 is activated in response to diverse stressors from different sources (left-side colored boxes) mediated by transcription factors (TFs), microRNAs (miRNAs), and additional signaling molecules (Sig. Mols.) (right-sided colored ovals). Posttranslational modifications (PTMs), chromatin states (ΔChromatin), feedback (curved arrow), and feed-forward (double straight arrows) regulatory loops and many additional processes mediate the activation state of HSF1. HSF1 binds to HSE consensus motifs at gene enhancer (HSE1), promoter (HSE2), and intronic (HSE3) sites to classically activate transcription of the heat shock protein 70 (*hsp70*) gene and numerous other genome-wide transcriptional targets that modulate developmental, metabolic, aging/longevity, and numerous additional pathways. (*Lower*) HSF1 and other stress-responsive transcription factors are frequently activated [A° (amplitude): subthreshold or threshold levels] at developmental critical periods during the prodromal phase of disease when cellular dysfunction is kept in check by compensatory/homeostatic mechanisms until the latter wane, coincident with increasing cellular dysfunction that results in the transition to manifest disease. Torii et al. (7) have developed different HSE-mediated fluorescence molecular constructs and associated mouse models to identify, follow, isolate, and interrogate stressed and vulnerable cells during the prodromal phase associated with transient threshold activation of HSF1 (RFP: red lines) and also during long-term tracing of descendant cells (GFP: continuous green line) at later stages of disease.

to various classes, strengths, combinatorial profiles, and reservoirs of diverse stressors. Previous work has defined the effects of a more limited set of perturbations or stressor states on stress-responsive regions of the brain and on defining stress-responsive regulatory processes that mediate signal efficacy and effector functions (8, 9). These studies have also emphasized that stress-responsive transcription factors participate in a dynamic array of transcriptional processes that mediate a wide variety of normal and pathological biological functions (9, 10). However, until now the potential to contextualize these biological properties arrayed in time and space and in relation to ongoing stress-mediated impairments and compensatory mechanisms underpinning normal physiological processes, disease vulnerabilities, and comorbidities have remained elusive.

Stress responses represent evolutionarily conserved mechanisms involved in normal physiological processes, and in response to disease states, genetic predispositions, and transgenerational influences (9). Stress-responsive transcription factors, microRNAs, and associated signaling molecules/pathways mediate these multipronged adaptations (9) (Fig. 1). Seminal stress factors, such as HSF1, orchestrate cellular homeostasis by differentially modulating expression profiles of genes that encode heat shock proteins, although they also dynamically reprogram diverse transcriptional responses during stress when global transcription is attenuated, translation is abrogated, and cell cycle progression is impeded (10). HSF1 also has prominent roles in development, metabolism, and aging/longevity. Through interrogation of these processes, we are beginning to gain insight into the coordination of gene expression among target cells, tissues/organs, and organismal responses to developmental and age-related processes in health and disease. HSF1 and other stress-responsive factors are modulated by DNA binding and protein–protein interactions, posttranslational modifications, protein degradation pathways, epigenetic mechanisms, posttranscriptional processing, intracellular localization, cell cycle regulation and metabolic states of effector cells, autoregulation and feed-forward signaling, cross-tolerance and cross-adaptation, as well as preconditioning (9, 10). Physiological stress responses during development and aging may be coordinately linked through neuronal/neural network response pathways by specific proneural and other (FOXO) transcription factors that preserve stem cell quiescence and the integrity of stem/progenitor pools, prevent loss of neuronal subtype identity/functions, maintain neuronal homeostasis and survival, and orchestrate cell type-specific and interorgan communications that promote adaptations associated with longevity (11).

These new molecular reagents and animal models, when used in concert with emerging combinatorial fluorescence labeling/imaging and gene-manipulation technologies, will also allow us to more fully

interrogate the importance of stress responses in recently identified, seminal but still poorly characterized biomedical processes. In disease states, these include the actions of: (*i*) early-stage proinvasion metastasis drivers (12); (*ii*) novel macromolecular complexes (the epichaperome and HSP90/HSC70-associated nucleating sites) mediating tumor survival (13); (*iii*) autophagy-mediated degradation of the nuclear lamina that attenuate oncogene-induced senescence (14); (*iv*) recurrent double-strand DNA breaks/replication stress-associated fragile sites in neural stem/progenitor cells that predispose to specific neural cancers (15); (*v*) epigenetically mediated repression of genes promoting adult neuronal cell maintenance and de-repression of genes mediating alternative neural cell subtypes that orchestrate neurodegeneration (16); (*vi*) genome-wide HSF1 transcriptional effects that modulate promoter-proximal pause release, gene-enhancer functions, and abrogation of neurodegenerative disease hallmarks (17); and (*vii*) enhanced developmental posttranslational modifications (DYRK1A protein kinase; Down's syndrome) normally predisposing to Alzheimer's disease that destabilize stress-responsive transcription factors (HIF2α) by inhibiting ID2–HIF2α feed-forward loops to abrogate glioma stem cell properties (inverse cancer comorbidity) (18). For physiological processes, these include the following: (*i*) rapid and selective nuclear export of gene transcripts encoding stress-responsive transcription factors without undergoing normal mRNA-mediated quality control (19); (*ii*) developmental stress signaling mediating cardinal maturational/maintenance cellular checkpoints [a differentiation checkpoint involving cross-talk between helix–loop–helix transcription factors and Hippo pathway signaling (20); a fitness checkpoint promoting organ integrity for cells exhibiting elevated levels of molecular/subcellular damage identified by alternatively spliced cell surface proteins and cell competition effects (21)]; and (*iii*) environmentally responsive neural codes for food abundance that modulate lifespan via stress resistance and metabolic reprogramming through the interplay of neuronal subtypes that encode food stores, metabolic states and stress responses (22).

Acknowledgments

M.F.M.'s research is supported by National Institute of Neurological Disorders and Stroke of the National Institutes of Health Grants R01 NS096144, R01 NS091519, and U10 NS086531.

1 Faa G, et al. (2016) Fetal programming of neuropsychiatric disorders. *Birth Defects Res C Embryo Today* 108(3):207–223.
2 Molero AE, et al. (2016) Selective expression of mutant huntingtin during development recapitulates characteristic features of Huntington's disease. *Proc Natl Acad Sci USA* 113(20):5736–5741.
3 Tabarés-Seisdedos R, et al. (2011) No paradox, no progress: Inverse cancer comorbidity in people with other complex diseases. *Lancet Oncol* 12(6):604–608.
4 Saxena S, Caroni P (2011) Selective neuronal vulnerability in neurodegenerative diseases: From stressor thresholds to degeneration. *Neuron* 71(1):35–48.
5 Shoshani O, Zipori D (2015) Stress as a fundamental theme in cell plasticity. *Biochim Biophys Acta* 1849(4):371–377.
6 Zhang F, Lupski JR (2015) Non-coding genetic variants in human disease. *Hum Mol Genet* 24(R1):R102–R110.
7 Torii M, et al. (2017) Detection of vulnerable neurons damaged by environmental insults in utero. *Proc Natl Acad Sci USA* 114:2367–2372.
8 McEwen BS, et al. (2015) Mechanisms of stress in the brain. *Nat Neurosci* 18(10):1353–1363.
9 Kagias K, Nehammer C, Pocock R (2012) Neuronal responses to physiological stress. *Front Genet* 3:222.
10 Mendillo ML, et al. (2012) HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell* 150(3):549–562.
11 Webb AE, et al. (2013) FOXO3 shares common targets with ASCL1 genome-wide and inhibits ASCL1-dependent neurogenesis. *Cell Reports* 4(3):477–491.
12 Hosseini H, et al. (2016) Early dissemination seeds metastasis in breast cancer. *Nature* 540:552–558.
13 Rodina A, et al. (2016) The epichaperome is an integrated chaperome network that facilitates tumour survival. *Nature* 538(7625):397–401.
14 Dou Z, et al. (2015) Autophagy mediates degradation of nuclear lamina. *Nature* 527(7576):105–109.
15 Wei PC, et al. (2016) Long neural genes harbor recurrent DNA break clusters in neural stem/progenitor cells. *Cell* 164(4):644–655.
16 von Schimmelmann M, et al. (2016) Polycomb repressive complex 2 (PRC2) silences genes responsible for neurodegeneration. *Nat Neurosci* 19(10):1321–1330.
17 Mahat DB, Salamanca HH, Duarte FM, Danko CG, Lis JT (2016) Mammalian heat shock response and mechanisms underlying its genome-wide transcriptional regulation. *Mol Cell* 62(1):63–78.
18 Lee SB, et al. (2016) An ID2-dependent mechanism for VHL inactivation in cancer. *Nature* 529(7585):172–177.
19 Zander G, et al. (2016) mRNA quality control is bypassed for immediate export of stress-responsive transcripts. *Nature* 540:593–596.
20 Wang LH, Baker NE (2015) E proteins and ID proteins: Helix-loop-helix partners in development and disease. *Dev Cell* 35(3):269–280.
21 Merino MM, et al. (2015) Elimination of unfit cells maintains tissue health and prolongs lifespan. *Cell* 160(3):461–476.
22 Entchev EV, et al. (2015) A gene-expression-based neural code for food abundance that modulates lifespan. *eLife* 4:e06259.