Adapting Proteostasis for Disease Intervention

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The protein components of eukaryotic cells face acute and chronic challenges to their integrity. Eukaryotic protein homeostasis, or proteostasis, enables healthy cell and organismal development and aging and protects against disease. Here, we describe the proteostasis network, a set of interacting activities that maintain the health of proteome and the organism. Deficiencies in proteostasis lead to many metabolic, oncological, neurodegenerative, and cardiovascular disorders. Small-molecule or biological proteostasis regulators that manipulate the concentration, conformation, quaternary structure, and/or the location of protein(s) have the potential to ameliorate some of the most challenging diseases of our era.

Proteostasis refers to controlling the concentration, conformation, binding interactions (quaternary structure), and location of individual proteins making up the proteome by readapting the innate biology of the cell, often through transcriptional and translational changes (Fig. 1). Proteostasis thus influences specific cellular functions and enables differentiated cells to change their physiology for successful organismal development and aging in the face of constant intrinsic and environmental challenges to prevent disease onset. Proteostasis is influenced by the chemistry of protein folding/misfolding and by numerous regulated networks of interacting and competing biological pathways (Fig. 1) that influence protein synthesis, folding, trafficking, disaggregation, and degradation (1–8). Herein, we examine a growing body of evidence suggesting that adapting the cellular proteostasis network by using "proteostasis regulators" (Fig. 1) can partially correct proteostatic deficiencies that contribute to a broad range of human diseases, some that present at birth, but most upon aging.

Proteostasis Maintenance During Development and Aging

The competition between cellular protein folding and degradation, often referred to as protein quality control, is one of many processes influencing proteostasis. The additional components of the proteostasis network (Fig. 1) are in place to achieve proteome maintenance with alternatives in addition to degradation. Protein folding in vivo is accomplished through interactions between the folding polypeptide chain and macromolecular cellular components, including multiple classes of chaperones and folding enzymes—interactions that minimize aggregation (Fig. 2) (3). Metabolic enzymes also influence cellular protein folding efficiency because the organic and inorganic solutes produced by a given compartment affect polypeptide chain solvation through noncovalent forces, including the hydrophobic effect, that influences the physical chemistry of folding. Metabolic pathways also produce small-molecule ligands that can bind to and stabilize the folded state of a specific protein(s), enhancing folding by shifting folding equilibria (9, 10). Whether a given protein folds in a certain cell type depends on the distribution, concentration, and subcellular localization of chaperones, folding enzymes, metabolites, and the like (3).

Temporal cellular proteostasis adaptation is necessary as a result of the presence of an ever-changing proteome during development and the presence of new proteins and the accumulation of misfolded proteins upon aging. Because the fidelity of the proteome is challenged during development and aging, and by exposure to pathogens that demand high protein folding and trafficking capacity, cells use stress sensors and inducible pathways to respond to a loss of proteostatic control. These include the heat shock response (HSR) (11) that regulates cytoplasmic proteostasis and the unfolded protein response (UPR) (2, 12) that helps maintain exocytic
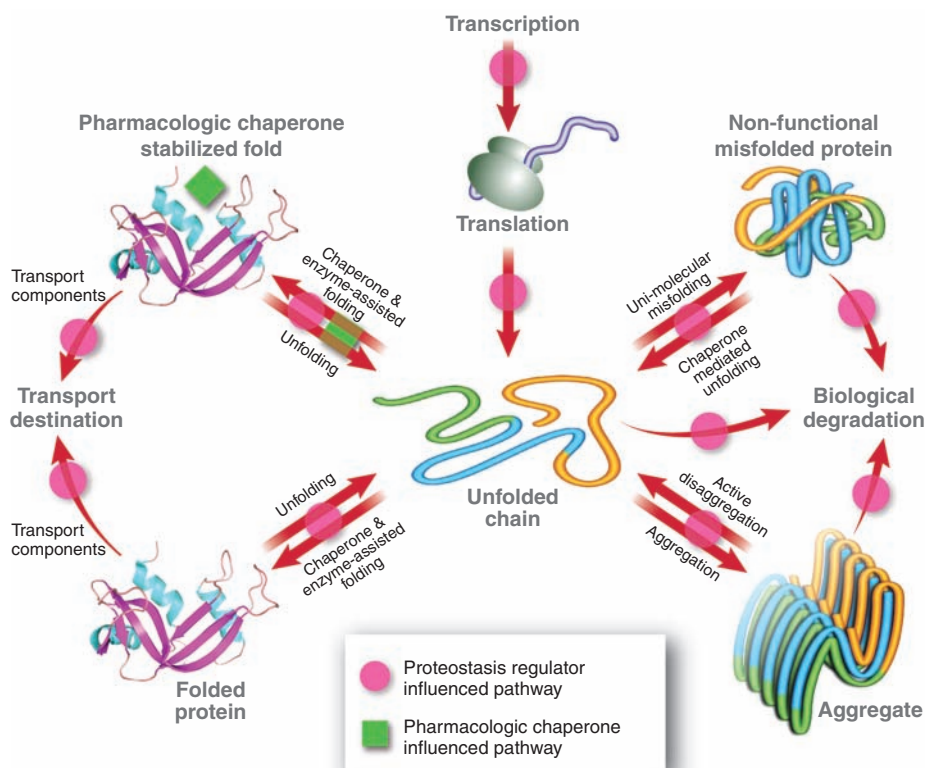

Fig. 1. A proteostasis network comprising pathways represented by the red arrows. Imbalances in proteostasis often lead to disease and, therefore, proteostasis regulators (magenta circles) that manipulate the proteostasis pathways/network can restore protein homeostasis and ameliorate both loss- and gain-of-function diseases. A finite population of the folded conformational ensemble is required for pharmacological chaperones (green squares) to enhance folding and trafficking, through a mechanism distinct from the innate biological pathways influenced by proteostasis regulators (ribonuclease A is shown; Protein Data Bank ID, 2BLP).

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pathway proteostasis. Recent experiments reveal that some cells possess a proteostasis maintenance capacity that can be exceeded when a new misfolding-prone protein appears (13). An age-associated decline in proteostatic control in concert with an increase in protein oxidation and modification that exacerbates aggregation challenges the maintenance of proteostasis during aging, offering a partial explanation for why many diseases are age onset (14–17). Much of the current knowledge about proteostasis results from studying cells under optimal growth conditions, but how proteostasis is accomplished in the face of nutritional or environmental challenges, especially upon aging, remains to be established.

Proteostasis Maintenance Is Linked to Healthy Aging

Misfolding-prone proteins challenge proteostasis within and outside the cell. The inability to restore proteostasis leads to diseases conveniently categorized as loss- or gain-of-function disorders (18). Loss-of-function diseases, including cystic fibrosis and Gaucher disease, are typically caused by inherited mutations leading to inefficient folding and excessive degradation (9, 19). Gain-of-toxic-function diseases, on the other hand, appear to arise when aggregation-associated proteotoxicity dominates over clearance inside and/or outside the cell (6, 18). The latter maladies, often associated with aging, include Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Alzheimer's disease. Emerging evidence suggests that proteostasis is controlled by signaling pathways that also influence longevity and youthfulness (Fig. 3) (6, 20–23). Therefore, treatments aimed at restoring proteostasis to alter the clinical course of multiple aging-associated disorders of complex etiology could also influence longevity. Emerging strategies to restore proteostasis in both loss- and gain-of-function diseases by manipulating the innate biology of the cell are outlined below.

Protein Replacement, the Current Therapeutic Strategy

Loss-of-function diseases are currently treated by intravenous administration of a wild-type version of the deficient protein to restore function. However, the scope of protein replacement therapy as a general solution is limited because the injected protein must find its way to the appropriate cell type and to the relevant subcellular compartment through an endocytic trafficking pathway. In enzyme replacement therapy for lysosomal storage diseases like Gaucher disease, less than 5% of the injected enzyme makes it to the target organelle (the lysosome). Although protein replacement therapy is effective for some loss-of-proteostasis diseases, alternative approaches are clearly necessary, especially for brain disorders, because recombinant proteins do not cross the blood-brain barrier.
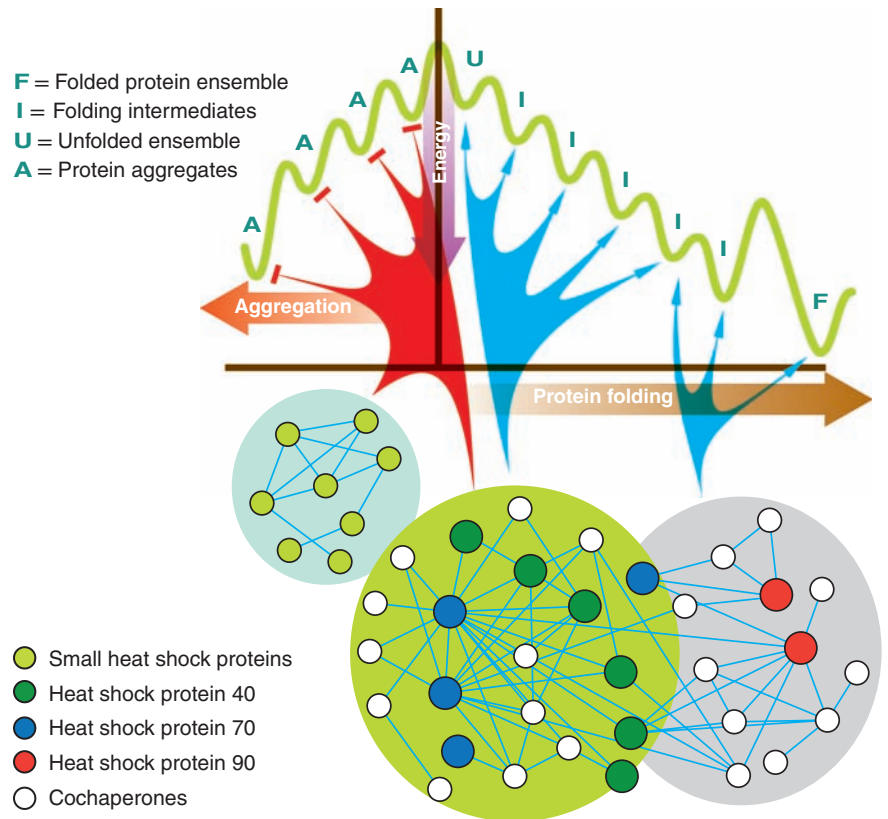

F = Folded protein ensemble
I = Folding intermediates
U = Unfolded ensemble
A = Protein aggregates

- Small heat shock proteins
- Heat shock protein 40
- Heat shock protein 70
- Heat shock protein 90
- Cochaperones

Fig. 2. The chaperone/cochaperone–assisted folding and aggregation prevention pathways, one component (red arrow) of the proteostasis network depicted in Fig. 1. Many chaperones and cochaperones interact with each other (depicted by the interaction map) and the polypeptide undergoing structure acquisition (indicated by the arrows to the structures on the folding free-energy coordinate) to produce a low-energy functional protein fold while avoiding aggregation.

Protein Stabilization to Remedy Loss- or Gain-of-Function Diseases

Two emerging therapeutic strategies offer promise to ameliorate gain- or loss-of-function diseases. For the latter, a so-called pharmacologic chaperone that binds to and stabilizes the folded, functional form of a mutant protein and shifts the folding equilibria away from degradation and aggregation may be useful (Fig. 1) (9). Pharmacologic chaperoning increases the concentration and proper localization of the protein by coupling more of the folded state to the trafficking pathway (Fig. 1), a pathway that strongly influences proteostasis (3). Pharmacologic chaperones are currently being evaluated in phase II clinical trials for Gaucher and Fabry diseases (24) and may prove useful for cystic fibrosis.

Small molecules that bind to the folded functional state of a protein can also impose kinetic stability on it, preventing its denaturation and misassembly into cytotoxic aggregates (10). These so-called "kinetic stabilizers" are currently being evaluated in placebo controlled clinical trials to stabilize transthyretin against amyloidogenesis associated with familial amyloid polyneuropathy, a gain-of-toxic-function disease (24).

Adapting the Proteostasis Network to Restore Normal Physiology

In contrast to the protein replacement and pharmacologic chaperone/kinetic stabilizer approaches, a more general therapeutic strategy to restore proteostasis may be to use small molecules or biologicals [small interfering RNA (siRNA), cDNA, or protein] to manipulate the concentration, conformation, and/or the location of a given protein or family of proteins by re-adapting the innate biology of the cell. This can be accomplished by altering the proteostasis network, including protein synthesis, folding, trafficking, disaggregation, and degradation pathways (Fig. 1) (1–8, 25–28). Proteostasis regulators often function by manipulating signaling pathways and/or the transcription and translation of components of a given pathway(s) composing the proteostasis network, including the HSR and UPR. Below we provide evidence that both loss- and gain-of-function diseases associated with defective proteostasis can be remedied with proteostasis regulators.

Proteostasis Regulators in Loss-of-Function Disease Cell Lines

Proteomic analysis of the chaperones required to fold the cystic fibrosis transmembrane conduct-

ance regulator (CFTR) chloride channel reveals that the ΔF508 CFTR cystic fibrosis–associated mutant is trapped as a folding intermediate in the endoplasmic reticulum (ER), sequestering more chaperones and cochaperones than wild-type CFTR (27). Readjusting the chaperone folding pathway with an siRNA proteostasis regulator directed against one cochaperone (Aha 1) restores partial folding, trafficking, and >50% of wild-type halide conductance, possibly by adjusting the Hsp-90/Aha-1 folding cycle to match the altered folding kinetics of ΔF508 CFTR (19, 27). Proteostasis regulators and pharmacologic chaperones are expected to act synergistically in cystic fibrosis, owing to their distinct mechanisms of action (Fig. 1).

Several small-molecule proteostasis regulators have recently been discovered that partially restore enzyme homeostasis in multiple cellular models of loss-of-function lysosomal storage diseases. The proteostasis regulators diltiazem and verapamil appear to function by inhibiting L-type Ca^{2+} channels in the plasma membrane, decreasing intracytoplasmic Ca^{2+} levels, which leads to increased transcription and translation of numerous cytoplasmic and ER chaperones, including BiP and Hsp40, that enhance the folding, trafficking, and activity of lysosomal enzymes (29). Thus, proteostasis regulators can augment the capacity of the cell to fold damaged proteins in both cystic fibrosis and lysosomal storage diseases (Figs. 1 and 2) (27, 29).

Ameliorating Gain-of-Toxic-Function in Organismal Disease Models

RNA interference (RNAi)–based proteostasis regulators that readjust disaggregase and chaperone levels, through manipulation of aging signaling pathways (Fig. 3) substantially delay the onset of the gain-of-toxicity phenotype in Caenorhabditis elegans models of Alzheimer's and Huntington's diseases (6, 20–23). Genetic and biochemical evidence implicates Aβ and polyglutamine aggregation as the cause of neurotoxicity in Alzheimer's and Huntington's diseases, respectively, through a mechanism(s) that remains obscure (30).

RNAi-induced reduction of insulin growth factor signaling (Fig. 3) allows the DAF-16 transcription factor to enter the nucleus, extending the life span of the Alzheimer's C. elegans model by a factor of almost 2 and substantially delaying age-onset aggregation-associated toxicity in this cytoplasmic Aβ1-42 aggregation model (6, 21). The HSF-1 transcriptome is also required for this protection and appears to predominantly regulate a disaggregation activity, while DAF-16 seems to regulate a backup active aggregation pathway that converts highly toxic Aβ oligomers into less toxic amyloid fibrils (6). Proteostasis regulators that enhance both the disaggregase and active aggregation pathways and/or related activities represent promising therapeutic strategies for gain-of-function proteotoxicity diseases (Figs. 1 and 3).

Huntington's disease is the prominent member of a family of gain-of-toxic-function diseases caused by an expansion of a contiguous polyglutamine tract beyond a threshold length, resulting in protein aggregation in the cytoplasm and neurotoxicity, with an age of onset that inversely correlates with the length of the polyglutamine repeat (20). RNAi directed against the proteostasis regulator age-1 (a phosphoinositide-3 kinase) in the insulin signaling pathway (Fig. 3) mediates slowing of the aging process and an increase in chaperone expression levels in Q_{82} expressing worms, conferring protection from proteotoxicity, and demonstrating that the adaptable biology of proteostasis can ameliorate polyQ proteotoxicity (20).

That the age-1 proteostasis regulator rebalances the chaperone network to restore proteostasis in the Huntington's disease worm model follows from observations that overexpressing certain chaperones, including Hsp70, Hsp40, and CCT, suppresses aggregation-associated proteotoxicity in numerous neurodegenerative disease models (31–35). Moreover, a number of small-molecule proteostasis regulators, including the natural product celastrol (25), function by activating HSF-1 (heat shock factor 1), leading to cytoplasmic chaperone up-regulation, lending support to the idea that it is possible to "naturally" rebalance proteostasis.

Restoring Proteostasis in Metabolic Diseases

Proteostasis defects that arise from ER folding deficiencies may be at the core of metabolic syndrome and type II diabetes (36). In the leptin-deficient ob/ob mouse model of severe obesity and
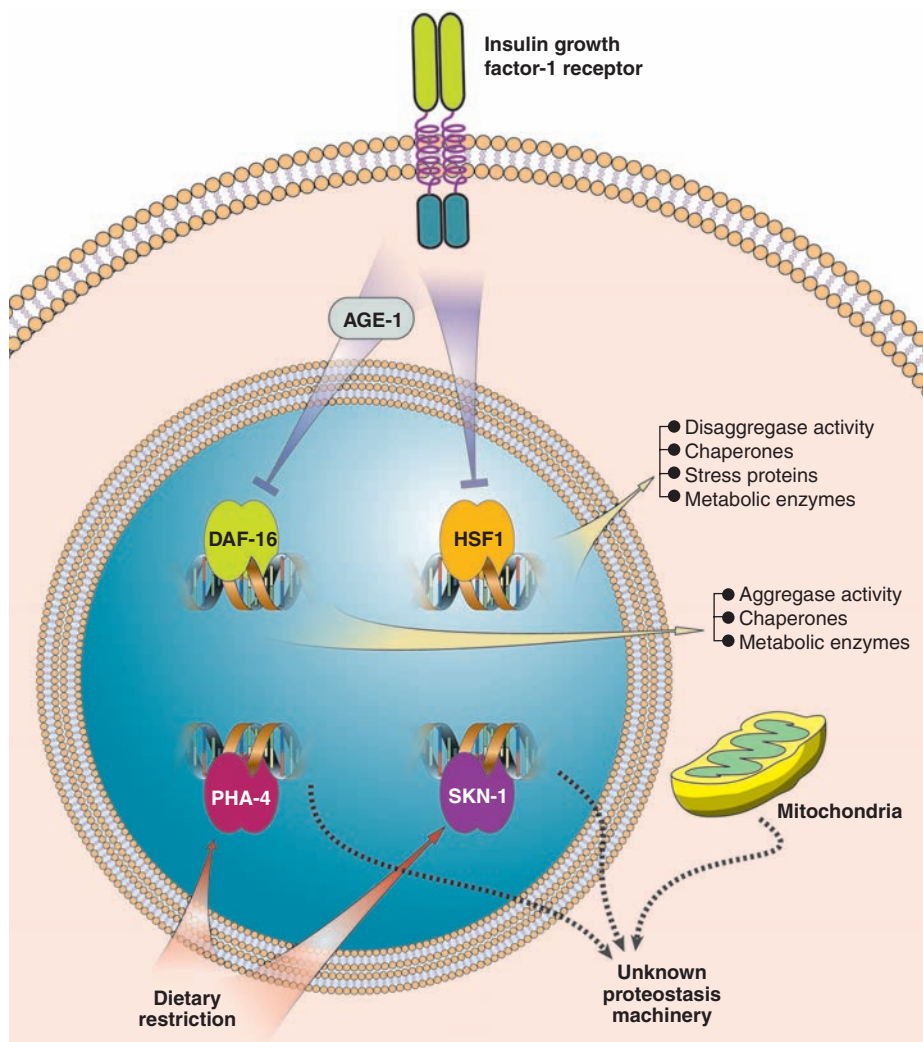

Fig. 3. Signaling pathways that control longevity and youthfulness strongly influence proteostasis. The insulin growth factor-1 receptor signaling pathway negatively regulates the activity of the transcription factors DAF-16 and HSF-1. HSF-1 regulates the transcription of stress response proteins, including chaperones, as well as a protein disaggregase activity. DAF-16 also mediates chaperone expression and appears to regulate an active aggregase activity. The dietary restriction pathway is known to suppress proteotoxicity in rodent models, which suggests that this pathway(s) (40, 41) also influences proteostasis. In a fourth mechanism, a decline in the flux through the mitochondrial electron transport chain results in extended life span; however, the links to proteostasis, if any, are unknown.

insulin resistance, the complex disease-associated traits are ameliorated by enhancing ER folding capacity using 4-phenylbutyrate (4-PBA) and taurine-conjugated ursodeoxycholic acid (*36*). Oral administration of these small molecules reversed hyperglycemia, increased glucose tolerance, decreased stress inside the ER in response to misfolding, and improved insulin receptor signaling. Notably, lipid accumulation in the liver also is resolved (*36*). 4-PBA has been used to partially restore the proteostasis of many misfolding-prone soluble and trans-membrane proteins traversing the exocytic pathway, including CFTR, where it is currently being tested in clinical trials to treat cystic fibrosis (*24*). Although the term "chemical chaperone" has been used as a catch-all category for compounds of unknown mechanism of action like 4-PBA (*37*) and compounds that clearly influence folding by acting as osmolytes, it is likely that molecules used in this study (including 4-PBA) fall in the proteostasis regulator category mechanistically, that is, they readjust the biological folding and trafficking machinery to increase insulin and insulin receptor folding and trafficking, known to play a major role in metabolic syndrome and type II diabetes.

Decreasing Folding/Trafficking Capacity as a Therapeutic Strategy for Infectious Diseases and Cancer

Manipulation of the proteostasis network by mammalian viruses represents an ancient requirement. Protein folding and trafficking down-regulation may prevent the occurrence of resistance in viral disease because enhanced folding and trafficking capacity is required for viral replication and assembly. For example, coadministration of Hsp90 inhibitors with antiviral drugs prevented the appearance of drug-resistant poliovirus strains in cell culture (*38*). Using proteostasis down-regulators that selectively target the folding of viral proteins or the synthesis of bacterial proteins that confer resistance may provide a general antiviral/antibacterial strategy by enabling control over the evolution of drug resistance (*38*).

A number of lines of evidence suggest that rapidly proliferating tumor cells require an increased protein folding and trafficking capacity to maintain basic physiology (*39*). Chaperone inhibitors, especially Hsp90 inhibitors, decrease cell proliferation in animal models, possibly by diminishing folding capacity, and are currently in phase II clinical trials for cancer (*24*). Thus, the facilitation of pathogen propagation and resistance, as well as proliferation of cancer cells by a high proteostasis capability, may be the principal reason that proteostasis capacity is closely matched to demand in most cells (*13, 39*), but this does not mean that restoring

the decreased proteostasis capacity in the aged to normal young-adult levels will predispose one to disease.

Slowing the Emergence of Complex Age-Onset Diseases

Aging challenges proteome homeostasis because of decreasing cellular proteostasis capacity and increasing protein damage (*14–17*). Given the central role of the proteome in physiology, proteostasis regulators may alleviate some of the defects facing the proteome of aged individuals and indirectly delay the onset of complex diseases of unknown etiology, such as a subset of autoimmune diseases and disorders like inclusion body myositis. Perhaps more intriguing is the possibility that restoring proteostasis capabilities to young-adult levels could reverse age-dependent imbalances that have very subtle phenotypes. In support of this idea, up-regulating proteostasis activities by the HSF-1 and DAF-16 transcription factors results in both an increased longevity of worms harboring misfolding-prone proteins and an increased ability to prevent aggregation-associated proteotoxicity (*6, 20*). Even if longevity is not increased in humans, restoring or maintaining proteostasis should increase the quality of life by delaying the onset and/or decreasing the impact of late-onset diseases.

Outlook

Alterations in the chemistry and biology of proteostasis, mediated by proteostasis regulators, are now established to ameliorate loss- and gain-of-function phenotypes in patient-derived cell lines or in organismal models of human diseases. The data show that the innate pathways composing the cellular proteostasis network can be rebalanced to slow the age-dependent decline in proteostatic control, preventing disease. Unlike pharmacological chaperones and kinetic stabilizers, which are protein and disease specific, one proteostasis regulator can reestablish proteostasis in multiple related diseases, as demonstrated by their efficacy in several lysosomal storage disease cell lines. Proteome repair, mediated by proteostasis regulators, has the potential to change the practice of medicine.

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