Disorders of Protein Misfolding: Alpha-1-Antitrypsin Deficiency as Prototype

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When taken together, three conceptual paradigms have led to major advances in understanding the clinical manifestations of protein misfolding and recently have led to novel therapeutic strategies. First, disorders caused by misfolded proteins are now classified according to the mechanism by which they cause clinical effects, either loss-of-function or toxic gain-of-function. Loss-of-function is the result of mutations that specifically alter folding, such that the function of the protein is impaired or such that the protein does not reach the cellular destination where its function is required, or both. Cystic fibrosis is the prototype disease caused by a loss-of-function mechanism in that all of the disease manifestations arise from lack of chloride transport. The CFTRΔF508 variant does not reach the apical surface of epithelial cells predominantly because of misfolding in the endoplasmic reticulum (ER) and rapid degradation by the proteasome. The small amount of CFTRΔF508 that does reach the cell surface is unstable and this probably also contributes to loss of chloride transport activity at epithelia. Toxic gain-of-function mechanisms are attributable to the pathologic activity of the mutant protein itself or to the effect of its mislocalization or both. This type of mechanism is implicated when the mutant protein produces a toxic effect in a cell line or live animal model. Huntington disease and early-onset forms of Alzheimer disease are prototypes of the gain-of-function mechanism as protein misfolding leads to degeneration of neurons. Diseases with childhood onset also fit into the paradigm, including conditions as diverse as respiratory failure in the newborn[1] and early-onset diabetes,[2] among many others.

A second paradigm has come from the recognition that cells possess multiple mechanisms by which they can respond to the consequences of protein misfolding, whether involving proteotoxicity and/or protein mislocalization. These mechanisms are now referred to as the proteostasis regulatory network.[3] The proteostasis network is constituted by systems that are designed to counteract proteotoxicity including chaperones to prevent misfolding and disposal pathways, such as the ubiquitin-dependent proteasomal pathway and the autophagic response, to degrade misfolded intermediates. The proteostasis machinery also includes signaling pathways such as the unfolded protein response and the heat shock response that alter the cellular transcriptome to counteract proteotoxicity by a broad series of mechanisms at multiple intracellular sites. Third, we have come to realize that disease caused by misfolded proteins reflects the net effect of the alteration in the protein together with the response of the cellular proteostasis network. For example, in gain-of-function diseases, clinical manifestations occur only when proteotoxic effects overwhelm the proteostasis network. This means that polymorphic variants in the proteostasis network may constitute genetic modifiers of the disease phenotype. Moreover, it means that drugs that enhance endogenous proteostasis mechanisms could theoretically prevent or delay the progression of clinical disease that is caused by proteotoxicity.

We will use alpha-1-antitrypsin deficiency (ATD) as a prototype of diseases caused by misfolded proteins and review recent findings about its pathobiology and the development of novel pharmacological strategies. The classic form of this deficiency is a relatively unique member of the protein misfolding diseases in that it causes disease in one target organ, chronic obstructive pulmonary disease (COPD), by loss-of-function and causes diseases in another target organ, hepatic fibrosis and carcinoma, by gain-of-function mechanism(s).

ATD Causes Target Organ Injury by Loss- and Gain-of-Function Mechanisms

The classic form of ATD is characterized by a point mutation that leads to misfolding of mutant α-1-antitrypsin Z (ATZ). ATZ accumulates in the ER of cells in which it is synthesized with reduced secretion such that serum levels are only 10%-15% of normal. Because liver is the predominant site of alpha-1-antitrypsin (AT) synthesis, accumulation of mutant ATZ within the ER of liver cells leads to proteotoxic

AT	Alpha-1-antitrypsin
ATD	Alpha-1-antitrypsin deficiency
ATM	Normal (wild type) alpha-1-antitrypsin
ATZ	Mutant alpha-1-antitrypsin Z
CBZ	Carbamazepine
COPD	Chronic obstructive pulmonary disease
ER	Endoplasmic reticulum
FDA	Federal Drug Administration
PBA	Phenylbutyric acid
RNAi	RNA interference
SNP	Single nucleotide polymorphism

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consequences, including hepatic fibrosis/cirrhosis and carcinogenesis, by gain-of-function.[4] In contrast, lung disease is predominantly caused by a loss-of-function mechanism. Because the major function of AT is inhibition of neutrophil proteases, including neutrophil elastase, cathepsin G, and proteinase 3, these enzymes are unchecked and degrade the extracellular matrix of the lung to cause COPD.[5]

In common with many other diseases of misfolded proteins, there is wide variability in the clinical phenotypes of ATD, implicating genetic and environmental modifiers as key determinants of disease prevalence and severity. Cigarette smoking markedly accelerates the rate and severity of COPD in ATD. It is believed that active oxygen intermediates that are released by mononuclear phagocytes in response to cigarette smoking functionally inactivate the small amount of AT that circulates in the blood, body fluids, and lungs in ATD. Even when this dominant environmental modifier is taken into consideration, there is evidence for variability in the lung phenotype of COPD that is thought to be attributable to genetic modifiers.[5] There is also marked variability in the liver disease phenotype. Studies of a unique cohort of individuals with ATD, identified by a nationwide newborn screening program in Sweden, have shown that only a subpopulation, 8%-10% of the cohort, develop liver disease over the first 40 years of life.[6] There is also a group of individuals with ATD who develop progressive liver disease later in life. Adults with liver disease attributable to ATD represent 85%-90% of the group that requires liver transplantation in the US.[7]

We have made a number of hypothetical predictions about the modifiers that determine heterogeneity in the hepatic phenotype of ATD. First, we suspect that several specific complements of modifiers determine several distinct types of childhood-onset liver disease in ATD: a small subset of children with ATD develop liver disease in the first year of life ("infantile" type); another group develop liver disease at 1 to 8 years of age ("childhood" type); portal hypertension may also first develop in adolescence ("adolescent" type). Other sets of modifiers determine adult-onset liver disease and these sets probably overlap with the modifiers that cause other types of age-dependent degenerative diseases that involve proteotoxic mechanisms. Second, we have theorized that these modifiers constitute the proteostasis regulatory network of the host and act by influencing the fate of mutant ATZ once it accumulates in the ER. Third, we have hypothesized that this proteostasis regulatory network predominantly works at 2 distinct levels, either by subtly altering intracellular degradative mechanisms or by modifying the signaling pathways that are designed to protect the cell from the consequences of the accumulation of aggregated protein in the ER (**Figure 1**). In terms of intracellular degradative mechanisms, we know that at least 2 systems are involved, the proteasomal and autophagic systems. Recent studies have shown that a Golgi-to-lysosome pathway mediated by sortilin also plays a role[8] and most likely there are other, as yet to be fully characterized, mechanisms that influence intracellular degradation of mutant ATZ. In terms of the putative signaling mechanisms, we know that accumulation of ATZ in the ER of cell line and mouse models activates a very distinct set of genes and signaling pathways, including, most prominently, autophagy and nuclear factor-kappaB (NFκB) but does not include the unfolded protein response.[9-11] In recent studies, we have found that the insulin signaling pathway also has a very powerful effect on ATZ accumulation and its proteotoxicity (O.S. Long, unpublished data, 2013). Other genes and signaling pathways that are activated in models of ATD, such as activation of ER- and mitochondrial-caspases and activation of transforming growth factor beta TGFβ signaling,[10-12] are believed to play a role in the type of hepatic damage that develops. The overall concept is that genetic variations in any of these mechanisms would lead to infantile, childhood, adolescent, or adult-type liver disease or protect the ATD individual from any clinically significant liver disease.

Autophagy appears to be particularly important in protecting cells and tissues from the proteotoxic effect of mutant ATZ accumulation in ATD. Autophagy is an ancient, conserved process induced by stress states, such as starvation, and involving the degradation of intracellular contents in lysosomes to generate new amino acids for survival. The process starts with formation in the cytoplasm of a double-membrane vesicle that envelopes cytosol and parts of subcellular organelles or entire organelles. This vesicle, termed autophagosome, eventually fuses with the lysosome, resulting in degradation of its contents and recycling of the amino acids that are generated. In cell line and mouse models of ATD, autophagy is activated by accumulation of ATZ in the ER and the autophagic response is responsible for degrading ATZ, particularly the insoluble polymerized/aggregated ATZ.[9,13] Recently, we found that an Federal Drug Administration (FDA)-approved drug, which enhances autophagic degradation of ATZ, carbamazepine (CBZ), reduces hepatic ATZ load and hepatic fibrosis in a mouse model of ATD.[14] This drug is now being tested in a clinical trial for severe liver disease attributable to ATD. Because autophagy is specialized for disposal of aggregation-prone proteins, drugs that capitalize on this endogenous proteostasis mechanism may be applicable to other age-dependent diseases involving aggregation-prone proteins and proteotoxicity. Indeed, the decline in autophagy with age has been implicated in the pathogenesis of Alzheimer's disease, cancer, cardiovascular disorders, inflammatory diseases, and glucose intolerance/metabolic syndrome.[15]

The theory that modifiers of the hepatic phenotype would target components of the proteostasis regulatory network was validated in a general way by early studies in which skin fibroblast cell lines from ATD patients with and without liver disease were engineered for expression of mutant ATZ. These studies showed that there is a lag in intracellular degradation of ATZ, specifically in ATD patients with severe liver disease.[16] However, there is still limited genetic proof of this theory. A single nucleotide polymorphism (SNP) in the downstream flanking region of ER mannosidase I has been