α1-antitrypsin Deficiency: A Misfolded Secretory Protein Variant with Unique Effects on the Endoplasmic Reticulum

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Abstract

In the classical form of α1-antitrypsin deficiency (ATD) a point mutation leads to accumulation of a misfolded secretory glycoprotein in the endoplasmic reticulum (ER) of liver cells and so ATD has come to be considered a prototypical ER storage disease. It is associated with two major types of clinical disorders, chronic obstructive pulmonary disease (COPD) by loss-of-function mechanisms and hepatic cirrhosis and carcinogenesis by gain-of-function mechanisms. The lung disease predominantly results from proteolytic damage to the pulmonary connective tissue matrix because of reduced levels of protease inhibitor activity of α1-anitrypsin (AT) in the circulating blood and body fluids. Cigarette smoking is a powerful disease-promoting modifier but other modifiers are known to exist because variation in the lung disease phenotype is still found in smoking and non-smoking homozygotes. The liver disease is highly likely to be caused by the proteotoxic effects of intracellular misfolded protein accumulation and a high degree of variation in the hepatic phenotype among affected homozygotes has been hypothetically attributed to genetic and environmental modifiers that alter proteostasis responses. Liver biopsies of homozygotes show intrahepatocytic inclusions with dilation and expansion of the ER and recent studies of iPS-derived hepatocyte-like cells from individuals with ATD indicate that the changes in the ER directly vary with the hepatic phenotype i.e there is much lesser alteration in the ER in cells derived from homozygotes that do not have clinically significant liver disease. From a signaling perspective, studies in mammalian cell line and animal models expressing the classical α1-antitrypsin Z variant (ATZ) have found that ER signaling is perturbed in a relatively unique way with powerful activation of autophagy and the NFκB pathway but relatively limited, if any, UPR signaling. It is still not known how much these unique structural and functional changes and the variation among affected homozygotes relate to the tendency of this variant to polymerize and aggregate and/or to the repertoire of proteostasis mechanisms that are activated.

Keywords

Autophagy; Proteostasis; ER storage

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Conflict of interest: Author states no conflict of interest

Introduction

ATD was first discovered in 3 patients with COPD when it first became possible to quantitatively analyze different globulin peaks in serum [1]. Over the years since then investigations have shown that AT is the most abundant protease inhibitor in serum and that it is predominantly produced by the liver. These two observations led to the protease-antiprotease imbalance theory for pathogenesis of COPD whereby lowering of antiprotease levels or activity rendered the lung connective tissue matrix susceptible to excessive proteolytic damage [2]. The protease-antiprotease imbalance theory was validated by the observation that cigarette smoking could functionally inactivate AT, via the effect of smoking on phagocyte-derived active oxygen intermediates [3]. This imbalance theory could thereby explain proteolytic attack on the lung connective tissue matrix in the vast majority of patients affected by COPD who were simply cigarette smokers and why patients with ATD had a marked increased in incidence and severity of COPD when they were also smokers.

Liver disease was first described in ATD by Sharp and colleagues who discovered it in a child with cirrhosis in 1969 [4]. Since that time ATD has been widely recognized as the most common genetic diagnosis for children who undergo liver transplantation. Eriksson and colleagues showed in 1986 that adults with ATD also had a predilection for cirrhosis and hepatocellular carcinoma [5], and onset of liver disease later in life is now known to be even more common than in childhood [6]. Wide variability in the incidence and severity of liver disease among individuals with the classical form of ATD was shown by analysis of a cohort of Swedish individuals identified in a nationwide screening study by Sveger [7,8]. Only ~8% of the cohort had clinically significant liver disease over the first 4 decades of life. A greater proportion of this population will probably be impacted as they reach older ages, but we know that a significant number of affected homozygotes completely escape clinical symptoms of liver disease throughout their lifetime [5].

Using a variety of model systems we have come to learn that the liver is damaged in ATD by gain-of-toxic function mechanisms activated by accumulation of misfolded ATZ within the early compartments of the secretory pathway [9]. Several theories for how this proteotoxicity leads to excessive collagen deposition and hyperproliferation in the liver have been proposed. Putative genetic and environmental modifiers are thought to determine susceptibility to and severity of liver disease among homozygotes [10]. We have postulated that these modifiers target proteostasis mechanisms that are designed to mitigate the proteotoxic effects of ATZ accumulation in the ER. Furthermore, we have postulated that these proteostasis mechanisms fall into two general categories: cellular pathways for degradation of misfolded ATZ and signaling mechanisms that permit cellular adaptation to the presence of misfolded ATZ [10]. Indeed, several novel concepts for therapeutic intervention that capitalize on the pathways for intracellular degradation of the ATZ variant are currently being investigated.

Clinical disorders

Although liver disease can be first diagnosed in infancy, childhood or adolescence, recent studies show that it is more common in adults with peak age 50–65 years [6]. More than

88% of all liver transplants done in the US for this diagnosis are adults. Some of these entries are not true homozygotes for the ATZ allele but, based on interrogating several different types of liver transplant databases, we ascertained that severe progressive liver disease occurs more than 2.5-fold more in adults than children with true homozygosity for ATZ [6]. Because the peak onset of 50–65 years correlates with the known age-dependent decline in autophagy and probably other proteostasis machinery, we suspect that the adult form of this liver disease is likely to reflect the normal aging process whereas onset in infancy, childhood and adolescence will be related to particularly powerful genetic and/or epigenetic modifiers.

The liver disease is predominantly characterized by fibrosis and ultimately cirrhosis. Recent studies have shown that the fibrotic response is characteristic of other protein accumulation disorders, including fibrosis in skeletal muscle in inclusion-body myositis, cardiac fibrosis in desminopathy and lung fibrosis in surfactant protein C deficiency and Hermansky-Pudlak syndrome [11–14]. Affected patients are also predisposed to hepatocellular carcinoma [5] and there is growing evidence that this pathological characteristic relates to heterogeneity in accumulation of misfolded ATZ among liver cells such that some cells evolve to a selective proliferative advantage and are trapped in a chronic hyperproliferative state [15].

The diagnostic hallmark of the disease is inclusions within some of the hepatocytes and these inclusions are known to contain ATZ but apparently not other proteins. These inclusions have long been characterized as representing rough ER. However, recent studies using iPS-derived hepatocyte like-cells and liver specimens from ATD patients indicate that the inclusions correspond to both ER and non-ER compartments [16]. The nature of the non-ER compartments is not yet known but they do not appear to be autophagosomes even though the liver cells have a marked increase in number of autophagosomes.

Destructive lung disease, COPD, does not appear clinically until adulthood. The peak age range is also fifth or sixth decade but very severe cases can be found in the late 20's or 30's [17]. Cigarette smoking is a powerful modifier of COPD and it is well established that AT can be functionally inactivated as a protease inhibitor by active oxygen intermediates, presumably released by phagocytes in response to cigarette smoking [3]. Nevertheless there are other modifiers of lung disease in ATD even when cigarette smoking is taken into consideration [17]. Recently we found evidence for fibrosis in the lungs of ATD patients who had undergone lung transplantation and in the PiZ mouse model of ATD [18]. Our data suggests that excess collagen deposition in the lung is caused by expression of the AT gene in respiratory epithelial cells and accumulation of the misfolded ATZ variant in pneumocytes can elicit fibrogenesis in the same way that it occurs in the liver in ATD and in the lung in other respiratory epithelial cell proteinopathies [13,14]. Modifiers that promote fibrogenesis could explain a subgroup of patients with ATD that have both lung and liver disease.

Mechanism by which the misfolded ATZ variant accumulates in the ER

The ATZ variant is characterized by a point mutation that substitutes lysine for glutamate 342 [9] and this leads to reduced secretion and intracellular accumulation of ATZ molecules [16, 19–22]. Immunostaining and biochemical analyses show that mutant ATZ accumulates

in early compartments of the secretory pathway, including most notably the rough ER, and perhaps other as yet undefined compartments that do not stain with classical markers of the rough ER [16]. It is important to note that in these model systems the secretion of ATZ is reduced whereas it is completely absent for a variety of null variants when they are expressed in similar model systems [23–25]. This reflects what is seen in humans with serum levels of ATZ at 10–15% of normal while those of the null variant are undetectable. Together, these observations make it possible to conclude that these model systems faithfully recapitulate the human disease and definitively prove that the primary abnormality is defective biogenesis. Furthermore, site-directed mutagenesis studies have shown that the single amino acid substitution of lysine for glutamate 342 is sufficient to cause the cellular defect in which the mutant ATZ is inefficiently transported through the intracellular secretory pathway [19].

It is still not entirely clear how the amino acid substitution leads to diminished secretion and intracellular accumulation of ATZ. Lomas and colleagues have argued that the primary defect is polymerization [26]. These authors demonstrated polymers and insoluble aggregates in liver biopsy specimens and plasma from patients with ATD and provided evidence for a loop-sheet insertion mechanism of polymerization. In this conceptualization of polymerization, the characteristic lysine substitution is at the hinge of the mobile reactive-site loop and because it has more bulk than the glutamate that is ordinarily at that site it prevents the reactive-site loop from relaxing into a known gap in the A sheet that is part of the flexible conformational changes of the AT molecule. This permits the reactive-site loop on another ATZ molecule to insert into the gap and begin the oligomerization and ultimately polymerization process. In an important study Sidhar et al showed that the secretory defect that characterizes ATZ was partially corrected by introducing a second mutation that suppressed polymerization [27]. More recent studies by Huntington and colleagues have suggested a different mechanism for polymerogenic and aggregation-prone properties of ATZ that involves at least 2 different domain-swapping phenomena (28–30) and this mechanism seemed most consistent with recent characterization of a putative ATZ monomer crystal structure [31].

Several lines of evidence suggest that misfolding is the primary defect responsible for impaired secretion/intracellular accumulation of ATZ and in this conceptualization polymerization/aggregation is a result rather than the primary defect itself. First, only 18% of the intracellular pool of ATZ is in polymers at steady state in a mammalian cell line model that faithfully recapitulates the intracellular accumulation/fate of ATZ [24]. Second, a naturally occurring variant of ATZ which has the same E342K mutation as ATZ and a carboxyl-terminal truncation accumulates in the ER even though it does not form polymers suggesting that misfolding is sufficient to lead to intracellular accumulation of ATZ (23). Third, the results of Sidhar et al [25] do not exclude the possibility that diminished secretion of ATZ is partially corrected by the second engineered mutation because this second mutation also prevents the primary misfolding defect. Furthermore, in a very interesting study a small molecule that prevents polymerization *in vitro* does not correct the secretory defect of ATZ *in vivo* but rather leads to enhanced degradation [32]. It is hard to know for certain about this applicability of this last study because there was no analysis of whether polymerization was prevented by the small molecule *in vivo*. Taken together, we believe that

misfolding is the primary defect and that polymerization and aggregation are time-dependent effects of the accumulation of ATZ that result from misfolding. This conceptualization is also consistent with the domain-swapping mechanism of polymerization described by Huntington in which polymerization is viewed as a 'kinetic' result, or delayed folding, of monomeric ATZ and explains how some ATZ gets secreted.

In one of the most interesting studies on the mechanism for ATZ accumulation in the ER, Nyfeler et al. provided evidence for the lectin ER Golgi intermediate compartment 53 kD protein (ERGIC-53) as an export receptor for AT [33]. Most importantly, ERGIC-53 failed to recognize mutant ATZ [33]. This study did not address whether polymerization prevents ATZ from being presented to ERGIC-53 or that the altered folding pathway of ATZ prevents an essential ligand domain from being available to bind to ERGIC-53 and there has been no follow-up of these important studies.

Mechanisms of intracellular degradation for the misfolded ATZ variant

Investigations of the mechanisms of ATZ degradation have shown that the proteasomal and autophagic pathways play critical roles. Early studies using yeast and mammalian cell lines showed that the proteasomal pathway participates in intracellular disposal of ATZ by a process that is now known as ER-associated degradation [ERAD] in which the substrate is extracted retrograde from the ER to the cytoplasm [34–37]. In fact, ATZ was one of the first identified substrates of the ERAD pathway [38].

Autophagy was then identified as a second major pathway for disposal of misfolded ATZ [39]. Autophagy is an intracellular catabolic pathway by which cells digest subcellular structures and cytoplasm to generate amino acids as a survival mechanism. It is characterized by double membrane vacuoles called autophagosomes which fuse with lysosomes for degradation of the internal constituents. Increased numbers of autophagosomes were observed in human fibroblast cell lines engineered for expression of mutant ATZ, in the liver of PiZ transgenic mice and in liver biopsy specimens from patients with ATD. Definitive evidence for the role of autophagy in ATZ disposal was provided by genetic studies in which ATZ disposal was delayed in autophagy-deficient [Atg5-null] murine embryonic fibroblast cell lines [40] and Atg6-null yeast strains [41,42]. We have also found that accumulation of ATZ within the ER and early vesicular compartments of the secretory pathway is sufficient to activate the autophagic response by demonstrating increased GFP+ autophagosomes in the liver of a transgenic mouse with liver-specific inducible expression of ATZ that had been mated to the (GFP-LC3) 'autophagosome reporter' mouse [40].

The importance of the autophagic pathway in intracellular ATZ degradation has been further validated recently by studies demonstrating that autophagy enhancer drugs promote intracellular ATZ disposal and attenuate hepatic fibrosis in the PiZ mouse model of ATD *in vivo* [43–45]. One of the concepts originating from studies of ATZ disposal in autophagy-deficient yeast strains is that autophagy becomes particularly important at higher levels of ATZ expression [41]. These results taken together with the structural constraints of the proteasome have led to the supposition that the proteasomal pathway degrades soluble

monomeric forms of ATZ whereas autophagy is needed for soluble and insoluble polymers. However, it is also possible that autophagy plays a role in the disposal of soluble monomeric ATZ that accumulates at levels of expression that exceed the capacity of the proteasome. Another important result from the studies by Kruse et al in autophagy-deficient yeast also showed that a misfolded fibrinogen variant associated with liver disease in a rare inherited form of hypofibrinogenemia is degraded by autophagy in a manner almost identical to that of misfolded ATZ [42].

Pathways for intracellular disposal of ATZ other than the proteasomal system and the canonical macroautophagy system are highly likely. For example, a sortilin-mediated pathway from Golgi to lysosome has been described to participate in degradation of ATZ in yeast and mammalian cell line models [41,46]. Another pathway for ATZ disposal which diverges from the canonical autophagy system was recently identified in a powerful *C. elegans* model of ATD and found to be present in a mammalian cell line model as well [22]. This pathway is particularly interesting because it is suppressed by insulin signaling and when up-regulated by knocking down components of the insulin signaling pathway it can completely mitigate ATZ proteotoxicity.

Signaling pathways activated by accumulation of the ATZ variant in the ER

To determine which signaling pathways are activated when ATZ accumulated in the ER, we developed cell line and mouse model systems with inducible rather than constitutive expression of ATZ because the latter would potentially permit adaptations that could obscure the primary signaling effects. A series of studies using these kinds of systems have shown that the autophagic response and the nuclear factor κB (NFκB) signaling pathway, but not the unfolded protein response, are activated when ATZ accumulates in the ER [47,48]. Activation of the autophagic response was shown by investigating the liver of a novel mouse model with hepatocyte-specific inducible expression of ATZ, the Z mouse, bred onto the GFP-LC3 mouse background. LC3 is an autophagosomal membrane-specific protein, so the GFP-LC3 mouse makes green fluorescent autophagosomes. Green fluorescent autophagosomes appear in the liver of the GFP-LC3 mouse only after 24 hours of starvation. In the Z × GFP-LC3 mouse green fluorescent autophagosomes are seen merely by allowing hepatocyte expression of the ATZ gene to be induced [40]. GFP+ autophagosomes are not seen in the liver of the Saar × GFP-LC3 mouse, which has hepatocyte-specific inducible expression of the AT Saar variant that accumulates in the ER but does not polymerize. Thus, autophagy is activated when ATZ accumulates in the ER and the autophagic pathway then plays a critical role in disposal of ATZ and in preventing massive intracellular aggregates.

Activation of NFκB is another hallmark of the cellular response to ATZ accumulation [48]. One of the most interesting aspects of the NFκB signaling pathway under these circumstances is that it is associated with a rather limited set of downstream transcriptional targets [49]. Indeed the most significant change in expression that could be attributable to NFκB is downregulation of Egr-1, a transcription factor that is essential for hepatocyte proliferation and the hepatic regenerative response [50]. Our most recent studies have indicated that the downregulation of Egr-1 in the liver of the Z mouse when ATZ expression is induced is directly attributable to the action of NFκB (A. Mukherjee and D. H. Perlmutter,

unpublished results). Furthermore, the complex of proteins that assembles to form NFκB when ATZ accumulates in the ER has a profile that is entirely distinct from that which forms when cells are treated with tumor necrosis factor (TNF) or tunicamycin (A. Mukherjee and D. H. Perlmutter, unpublished results). Finally, mating of the PiZ mouse to a mouse model with conditional hepatocyte-specific deficiency of NFκB activity shows more severe inflammation, fibrosis, steatosis, dysplasia, and more hepatocytes with globules (A. Mukherjee, T. Hidvegi and D. H. Perlmutter, unpublished results), indicating that NFκB signaling is intended to protect the liver from the effects of ATZ accumulation. Together, the data on NFκB suggest that it plays a particularly important role in the effects of ATZ on cell proliferation, survival, and ultimately the predisposition to hepatic carcinogenesis in AT deficiency.

Although some studies have suggested otherwise, most have been unable to demonstrate changes in gene expression indicative of an unfolded protein response (UPR) in systems characterized by intracellular ATZ accumulation [48]. Even when care is taken to eliminate the potential for cellular adaptation to ATZ accumulation by generating inducible expression of the ATZ molecule, there is negligible change in expression of the downstream targets of the UPR or the changes in expression are minimal compared to positive controls [49]. This means that activation of autophagy and NFκB in cells that accumulate mutant ATZ is independent of the UPR, another distinct characteristic of the cellular response in the AT deficiency state. With what is known about the mechanism by which the UPR is initiated it has always been relatively easy to understand how polymerized and aggregated ATZ would not elicit the UPR. Results of recent structural studies by Huntington and colleagues provide an explanation for why soluble monomeric ATZ does not get recognized by the UPR apparatus. Those results suggest that the monomeric ATZ intermediate adopts a conformation that resembles the wild-type molecule [29] and therefore would not be recognized as unfolded.

Transcriptomic analysis of liver when ATZ is induced in the Z mouse has identified other changes in gene expression that are attributable to the cellular response to ATZ [49]. One of these changes, upregulation of the regulator of G signaling 16 (RGS16), may represent a mechanism by which autophagy is activated in the liver in AT deficiency. We have found that the RGS16 response is characteristic and specific for ATZ. Because RGS16 anatagonizes Gαi3, and Gαi3 plays a role in inhibiting hepatic autophagy [51], we have hypothesized that increased RGS16 when ATZ accumulates in the ER leads to reversal of the inhibition of autophagy that would otherwise pertain in resting hepatocytes. There is still relatively limited information about how RGS16 is upregulated and where in the cell it acts to antagonize Gαi3.

The hepatic transcriptomic analysis of the Z mouse also shows changes in gene expression indicative of TGFβ signaling and this is consistent with the fibrotic response that represents the dominant pathological characteristics of the liver in ATD. Furthermore we have recently found that accumulation of the ATZ variant in respiratory epithelial cells elicits fibrosis in the lungs with evidence for fibrosis in the lungs of ATD patients with very severe COPD [18]. The mechanism by which accumulation of misfolded proteins in the ER elicits TGFβ signaling has not been studied. Other proteinopathies are known to elicit a fibrotic response

including inclusion-body myositis causing fibrosis in skeletal muscle [11], desminopathy causing cardiac fibrosis [12] and lung fibrosis in several rare proteinopathies that affect respiratory epithelial cells [13,14].

Taken together, these studies show a unique repertoire of signaling pathways activated by accumulation of the ATZ variant in the ER in model systems and, in many cases, validated by investigations of tissue from ATD patients. The implication of these results is that the substrate which accumulates elicits specificity in the response of the ER. In the case of the ATZ variant, there is a relatively limited, if any, role of the UPR signaling pathway and thus this ER storage disease represents an important example of why the UPR should be regarded as one of at least several responses to ER stress.

Structural changes in the ER in ATD

There is very little known about how accumulation of the ATZ variant affects the ER and elicits structural changes. Early ultrastructural studies of livers from patients with ATD showed dilation of ER with proteinaceous material in the lumen [52,53]. Mostly rough ER was described but these studies were done before reagents were available for marker studies or biochemical assays. Several observations have come from our recent studies of iPS-derived hepatocytelike cells from patients with ATD and those investigations led us to look again at liver from ATD patients [16]. First, we could conclude from endoglycosidase H digestion assays that ATZ predominantly localizes to pre-Golgi compartments. Second, double-label immunofluorescent analyses showed that ATZ accumulates in rough ER but also in one or more compartment that could not be considered rough ER. Ultrastructural studies showed markedly dilated rough ER in many cells but also there were very large vesicular structures enveloping proteinaceous material and only partially covered by ribosomes. These very large vesicular structures are probably what have been traditionally described as the globular inclusions that are the hallmark of ATD. The proteinaceous material in the vesicular structures appeared electron-dense in some areas. In some of the cells these vesicular structures were almost completely devoid of ribosomes but, because of the single plane of the image, it was not possible to exclude the presence of ribosome-containing areas. These observations led us to believe that there are structural changes other than simply dilation of the rough ER, perhaps including dilation of smooth ER, specialized subdomains of the rough ER or completely separate subcompartments as has been observed for other misfolded proteins [54].

Variations in disease incidence and severity---the case for proteostasis mechanisms

A number of years ago we, and others, hypothesized [9,10] that genetic and/or environmental modifiers could explain the differences in onset and severity of liver disease among Z homozygotes that were first recognized by the screening/cohort studies of Sveger [7,8]. Furthermore it was proposed that these modifiers would target proteostasis mechanisms, such as intracellular degradation pathways (Figure 1). This hypothetical paradigm was initially validated by studies showing that ATZ was degraded more slowly in ATD individuals with liver disease than in ATD individuals without apparent liver disease,

using skin fibroblast line models engineered for expression of ATZ by retroviral-mediated gene transfer [21]. Recently studies by Tafalang et al have provided further validation of this hypothesis by showing slower degradation of ATZ in iPS-derived hepatocytes (iHeps) from ATD individuals with liver disease as compared to iHeps from ATD individuals without liver disease [16]. Interestingly, the rates of ATZ degradation in iHeps were almost identical to those in skin fibroblasts published 20 years ago with a half-time of disappearance of ~ 4 hours in those affected by liver disease compared to ~2 hours in those without apparent liver disease. Furthermore, Tafaleng et al found that large intracellular globular inclusions were only seen in the iHeps of the liver disease patients (Figure 2).

These results are very important in several respects. First they validate the hypothetical paradigm that modifiers of disease target proteostasis mechanisms by showing that degradation may be relatively impaired in some ATD individuals with liver disease. This impairment can be demonstrated in iHeps kinetically and morphologically. Second, the results show that cell biological mechanisms can explain personalized variations in the clinical effects of a single gene defect and now we can explore the cell biological basis for sequence variants in putative modifier genes. Third, the results suggest the iHeps may be used to make pre-morbid predictions of liver disease susceptibility.

Studies designed to identify genetic and environmental modifiers of the liver disease phenotype in human populations have begun to appear in the literature in recent years. In one interesting study, a single nucleotide polymorphism (SNP) in the *MAN1B1* gene was found to be over-represented statistically in a series of infants with end-stage liver disease [55]. The variant was shown to reduce intracellular levels of the mannosidase [56]. Recent experiments have shown that Man1B1 is actually localized to the Golgi but it plays a role in regulation of protein secretion as a part of the protein quality control network which is recently recognized to be localized in the Golgi [57]. Furthermore those experiments have provided a basis for how reduced levels of Man1B1 could theoretically lead to greater intracellular ATZ accumulation [57]. These results for the Man1B1 variant would appear to validate our hypothesis that intracellular degradation pathways are targets of liver disease modifiers but further populations studies of this variant would be reassuring [58].

A SNP in the upstream flanking region of the AT gene has also been implicated in susceptibility to liver disease [59]. However, the nature of variant could not be reconciled with how it might affect liver disease susceptibility and its statistical association with variation in the liver disease phenotype was dependent on a questionable classification of population sub-groups.

Our hypothesis for variation in liver disease susceptibility also identifies signaling pathways that could increase or decrease ATZ proteotoxicity as potential targets for disease modifiers (Figure 1). As of yet we have not encountered an example of this potential scenario, but we predict that further studies of iHeps from patients with different forms of ATD liver disease, in terms of age of onset and type of hepatic pathology, will identify such a mechanism in the near future.

Concluding remarks

In the classical form of ATD a misfolded variant accumulates in the early part of the vesicular secretory pathway and causes liver disease by gain-of-toxic function. The site of accumulation includes rough ER and pre-Golgi compartment(s) that are not characteristic of rough ER. The misfolded variant has a tendency to polymerize/aggregate and that tendency appears to play an important role in the unique structural and functional consequences. The liver disease is characterized by fibrosis and hyperproliferation but it is variable in how it affects each host. There is growing evidence that this variability is due to genetic and/or environmental modifiers and that the modifiers act on proteostasis mechanisms that are unique for this misfolded variant. These proteostasis mechanisms include the proteosomal and autophagy degradative pathways and a repertoire of signaling pathways that does not include a full-fledged UPR but is characterized by activation of autophagy, NFκB and TGFβ signaling in distinct ways. Because of this we believe it is important to think of the UPR as one form of ER stress and that there is substrate specificity in how the ER responds to misfolded protein accumulation. Exciting new studies using iPS-derived hepatocyte-like cell line have provided initial evidence that we can deduce the personalized variations and how they affect the structure and function of the ER and other cellular machineries involved in protein quality control.

Acknowledgments

Abbreviations

AT	α1-antitrypsin
ATZ	α1-antitrypsin variant Z
ATD	α1-antitrypsin deficiency
COPD	chronic obstructive pulmonary disease
ER	endoplasmic reticulum
ERGIC-53	endoplasmic reticulum Golgi-intermediate compartment 53-kD protein
ERAD	endoplasmic reticulum-associated degradation
iPS	induced pluripotent stem cell
NFκB	nuclear factor κB
RGS16	regulator of G signaling 16
SNP	single nucleotide polymorphism
TGFβ	transforming growth factor β
UPR	unfolded protein response

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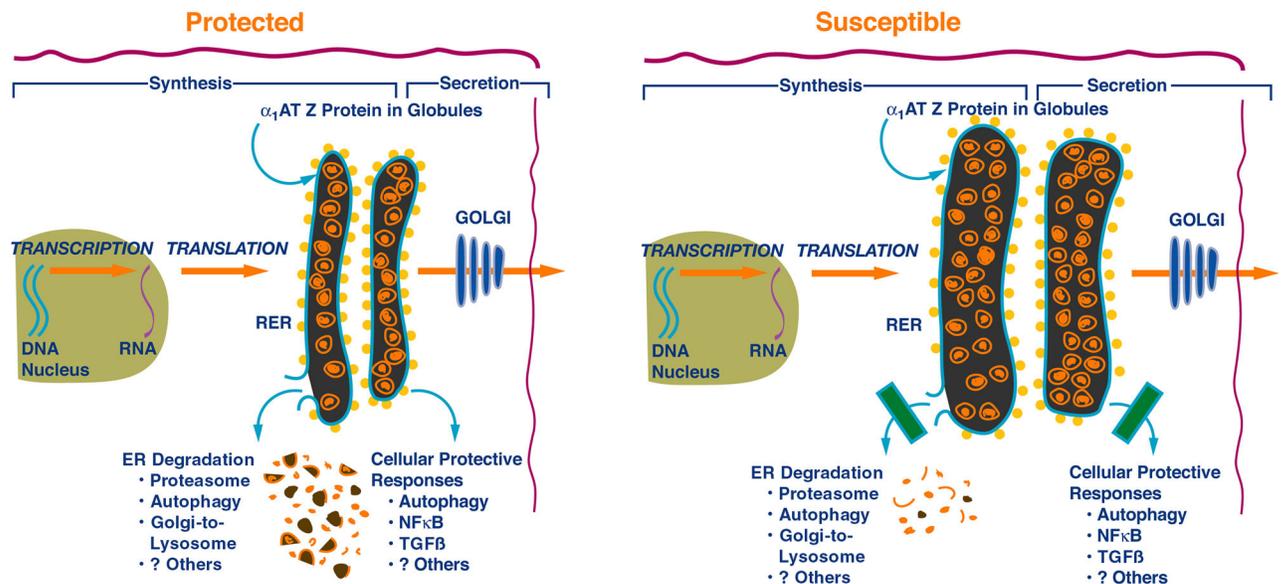
Figure 1.
Conceptual model for proteotoxicity in ATD. Liver cells from a putative protected host (left) can mitigate proteotoxicity by the action of ER degradation pathways (lower left) or putative protective cellular response pathways (lower right). In cells from a susceptible host (right) a subtle block (green bar) in either ER degradation pathways and/or the cellular response pathways leads to greater accumulation/proteotoxicity and chronic hepatic disease.

Induced pluripotent stem cells model personalized variations in liver disease due to α1-antitrypsin deficiency
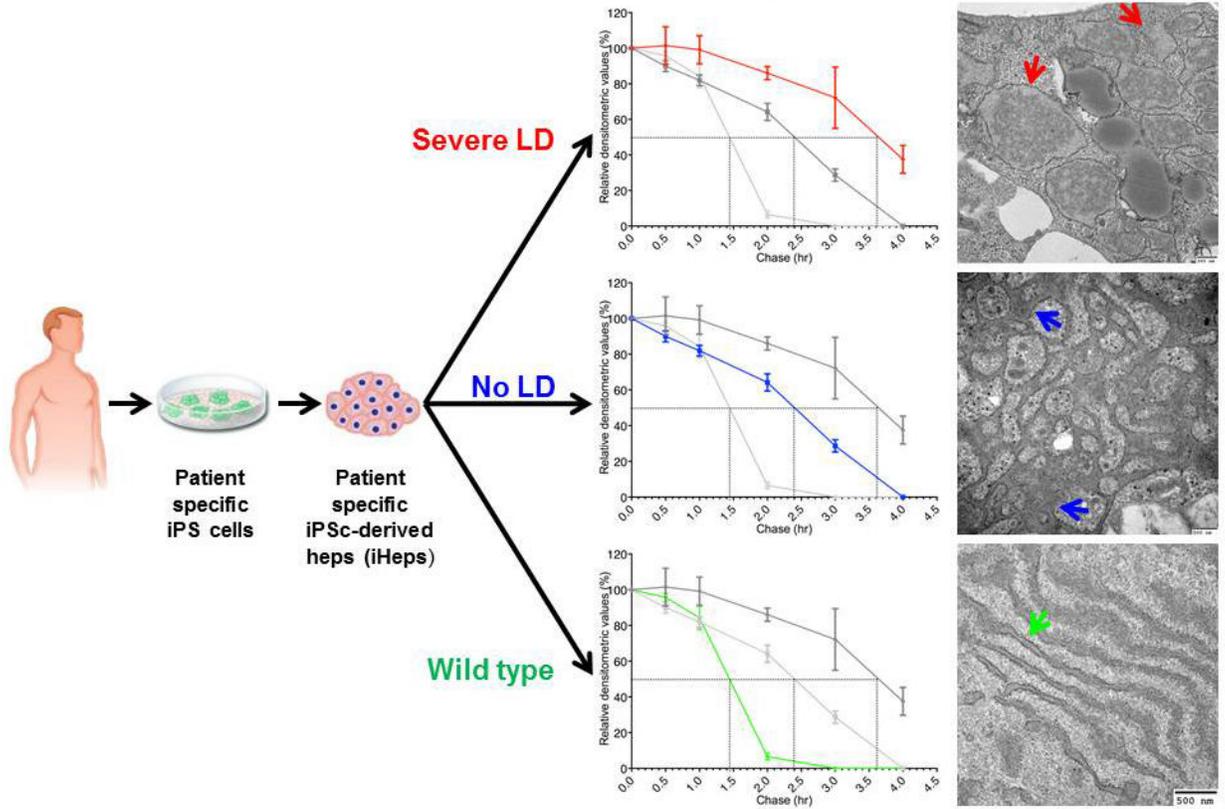

Figure 2.
Induced pluripotent stem cells (iPS) model personalized variations in disease due to ATD. iPS cells from ATD patients with severe liver disease (severe LD), no liver disease (no LD) or wild type were tested by pulse-chase (center) and ultrastructural (right) studies. For the pulse-chase studies the disappearance of ATZ-specific radioactivity as measured by densitometric scanning of SDS-PAGE gels is shown as a function of chase (hrs). The results show that for severe liver disease there is slower disappearance of ATZ and massive inclusions (red arrows) compared to wild type with faster disappearance and normal ER morphology without inclusions (green arrow). For no liver disease the disappearance rate is intermediate and there is dilated ER (blue arrow) but no massive inclusions. The figure was created and kindly provided by Drs Edgar Tafaleng and Ira Fox.