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Innovations

Probing the Proteostasis Network

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Alzheimer's disease (AD), Parkinson's disease (PD), cystic fibrosis (CF): all devastating diseases with virtually no effective treatments. While some companies are digging deeper into the genetic bases for these diseases, one Cambridge, MA, based company is going the other way. Rather than spiraling the research microscope down into ever smaller layers of biology, Proteostasis Therapeutics, Inc., (PTI) is taking a macro view. They are interrogating the biological causes of diseases by assessing the overall functionality of proteins in relation to their environments, a concept known as protein homeostasis.

The history of protein research in large pharma and biotech has mostly been focused on a target protein including enzymes and antibodies. "But if you think about that space of biology between the birth of a protein and the final fully folded, fully assembled functional protein, all of that biology in between is really the remit of proteostasis," explains Peter Reinhart, Ph.D., PTI's CEO.

"If you do not embrace the system, you are unlikely to be successful." —Jeffery Kelly

Targeting 10–12 Key Cellular Pathways

PTI is interested in cellular pathways and mechanisms involved in maintaining protein homeostasis. "We are interested in what allows proteins to fold properly, the cellular processes that get rid of misfolded proteins, and the protein degradation pathways that recognize when things are not right and degrade them," explains Reinhart, who remains an associate professor of neuroscience at Duke Medical Center. The idea of the company stems from the goal of trying to improve the proteostasis milieu of the cell by modulating key pathways. In the area of neurodegenerative or protein misfolding diseases in particular, interfering with disease-modulating pathways is the therapeutic focus.

Reinhart estimates that the proteostasis network consists of a collection of 1000–1500 key proteins organized into 10–12 pathways. Each pathway contains about 50–200 proteins. "There are not an infinite number of pathways," says Reinhart, "and they are all connected and have a lot of crosstalk between them." Further, the proteins within a pathway are highly interconnected, forming protein/protein interactions. Modulating one protein or one pathway has flow-on effects onto other pathways. "That is part of the reason for thinking of this overall group of proteins as a network, the proteostasis network that maintains the remainder of the proteome under constantly changing conditions."

The proteostasis network pathways have a common objective: keeping all of the proteins in a cell folded appropriately and trafficked to the appropriate place, which is accomplished by balancing protein production and degradation. Yet this system does not have a lot of reserve capacity. If the system is stressed, even a little bit, either in the context of environmental stress or genetic stress in the form of mutations, these stresses can very quickly overcomes the reserve capacity of the cell. Explains Reinhart, "The consequences include the buildup of misfolded proteins that then can form aggregates that then go on to form a number of the most significant diseases that we are dealing with in this generation." These include protein and aggregation disorders, like AD, PD, and Huntington's diseases (HD). Maintaining proteostasis is also related to misfolding and mistrafficking disorders, including the 50+ lysosomal storage diseases. It also includes CF, which results when the cystic fibrosis transmembrane conductance regulator (CFTR) protein misfolds in the endoplasmic reticulum and is degraded instead of making it out to the surface of the airway cells.

"We may not know exactly what contributes to the etiology of AD, for example, but if we can modulate a pathway that improves the cell's capability to remove toxic aggregates, such as the hyperphosporylated τ protein or soluble amyloid β oligomers, even in the absence of knowing how they are generated and their precise mechanism of cytotoxicity, we think we can produce a beneficial therapeutic outcome," says Reinhart. This approach is in contrast to much of the Alzheimer's therapeutic strategies that have gone into investigating inhibitors of the enzymes involved in creating these toxic entities, such as β secretase inhibitors, γ secretase inhibitors, or antibodies that mop up toxic amyloid β molecules.

Though they are not divulging the precise pathways under study, PTI is honing in on several groups of proteins and associated pathways. Stress-responsive signaling pathways are the means by which cells constantly monitor the integrity of protein homeostasis in a given organelle or subcellular compartment and then respond accordingly. These pathways include the heat-shock response, the unfolded protein response, and the mitochondrial unfolded protein response, all signaling cascades that work similarly to adjust proteostasis network capacity with demand. For example, when the heat-shock response is activated, several hundred members of the proteostasis network increase in concentration.

Other pathways involve chaperone protein- and enzyme-mediated pathways; they help proteins fold and traffic appropriately while degrading terminally misfolded proteins. Some of these pathways are enhanced by the unfolded protein response. Activation of protein degradation pathways, such as autophagy or modulation of the proteasome, are also high on PTI's list, especially for aggregation-associated degenerative diseases. "We are focusing on pathways

that can produce a beneficial outcome,'' says Reinhart. ''By shepherding proteins through various stages, either getting rid of them when they are misfolded or trafficking them more appropriately when they are stuck in an organelle (such as in CF), we hope to achieve a beneficial outcome in the context of the disease.''

Scientific Founders

The core science behind PTI came from the labs of its three scientific founders: Jeffery Kelly (Scripps Research Institute), Andrew Dillin (Salk Institute), and Richard Morimoto (Northwestern University). In 2000, when Kelly starting hearing other researchers talk about overexpressing individual chaperone proteins as an approach to fix a given misfolding or aggregation-prone protein, he remembered thinking this approach would be less efficient or fail because chaperones work as part of a complex system. ''If you do not embrace the system, you are unlikely to be successful,'' he says. Over the next 5 years, Kelly invested in learning more about the proteostasis network. He and his colleagues did their first technology-enabling experiments in the early 2000s, which led to the proof of principle that a small molecule could be useful to restore protein homeostasis.

''The trick is to match proteostasis network capacity with demand,'' says Kelly. ''And if you have a system that can do that, you can fix a misfolding-prone protein, for example, and render the protein functional.''

Kelly says there is mounting evidence that the inability to effectively signal through stress-responsive signaling pathways could play a role in neurodegenerative diseases. In animal models of these diseases, as the animals age, their ability to match healthy protein capacity with demands diminishes. ''The feeling is that even though this is true at the cell or organism level, you can still activate those pathways chemically,'' adds Kelly.

In the context of Alzheimer's disease, where the aggregation of the amyloid β peptide is linked to neurodegeneration, the Dillin and Kelly labs have shown that when heat-shock response signaling is activated, the pathological phenotype is reduced in both *C. elegans* and mouse models. Rick Morimoto's lab showed the same result in HD. Intriguingly, though the plaque numbers increase, the Alzheimer's animals show less neuronal loss and less behavioral and memory deficiencies. ''We argue that plaque formation is a response of the organism to protect itself,'' says Kelly. ''This is just some of the evidence that one can manipulate the networks and at least delay, if not prevent, neurodegeneration,'' says Kelly, who explains that animals treated in this way live a normal life span.

PTI set up operations approximately 18 months ago, backed by $45 million in venture capital funding. Though not necessarily restricting itself to small molecules, the company has put a lot of effort into discovering small molecules that activate a particular protein homeostasis network pathway deficient in a given disease. Today, its strategy is to connect small molecules to pathways that are known to remedy either misfolding diseases (associated with protein loss of function) or aggregation diseases (associated with protein degradation and gain of toxic function). ''We very much want to understand what components of the protein homeostasis are the best to target for each disease type,'' adds Kelly.

If this idea sounds vaguely familiar, but in another context, there is good reason: Kelly was a cofounder of FoldRx, a biotech company focusing on developing therapies for protein misfolding disorders, which was acquired by Pfizer in October 2010. Amicus Therapeutics, based in Cranbury, NJ, is also in the race to develop small molecule mutant enzyme stabilizers for specific lysosomal storage disorders.

Small Molecules as Proteostasis Modulators

PTI has built itself up as an entity that can test the concept of proteostasis modulators. The company is using its capabilities in transcriptional, proteomic, and functional profiling to probe the proteostasis network in human diseases. ''The goal of all of this work is to increase the health span, the number of years we are healthy,'' says Kelly.

To date, PTI's scientists have completed two high throughput screens totaling 1.25 million compounds to identify new chemical series. The company's chemistry group optimizes the chemistry and does standard medicinal chemistry. ''The only difference being that the optimization is done to optimize the state of the network modulation, as opposed to a single IC50 value, which is the way most large pharma optimizes chemistry,'' says Reinhart. PTI's functional biology group tracks functional efficacy in disease-relevant cell-based models and in human samples; its pharmacology group follows efficacy in animal-based models.

Though a human clinical trial of any new chemical compound is at least 2 years off, PTI eventually hopes to bring benefit to the larger neurodegenerative diseases like AD and PD. They are also honing in on retinal diseases, in particular diabetic retinopathy, and smaller orphan diseases.

''We are looking at controlling proteome function much in the same way enzyme inhibitors have been used historically,'' says Kelly. In some diseases, less protein or less active protein is the goal; for example, matrix metalloproteases are linked with cancer. Kelly theorizes cancer and other diseases could be helped by lowering protein-folding efficiency. ''Beyond misfolding and degenerative diseases, there is a much bigger picture in terms of controlling proteome function.'' PTI is out in front to ultimately prove that concept in the clinic.

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