DNAJ proteins: more than just “co-chaperones”

Kakkar, Vaishali

Chapter 1

AIM AND OUTLINE OF THE THESIS

Protein aggregates hallmark almost all the major neurodegenerative diseases known today, suggesting that an imbalance the maintenance of protein homeostasis underlies these diseases. The aim of this research project was to understand the role of heat shock proteins (HSP) in diseases associated with protein aggregates. **Chapter 2** provides a general overview of the various HSPs on how they function in the cells in situations of global acute proteotoxic stress and how this may differ from condition in which cells are exposed chronically to specific misfolded proteins for example in different forms of heritable neurodegenerative diseases. In this chapter, we tried to come up with a first type of "barcoding" of each of these diseases with those HSPs that were experimentally suggested to counteract the aggregation of the disease-causing proteins, providing some initial understanding of HSP specificities in different diseases. Important for this thesis, members of the DNAJ protein family repeatedly are found to be able to delay aggregation of the disease-causing proteins and in doing so generally act in collaboration with members of the Hsp70 family.

Chapter 3 next deals with two members of the DNAJ superfamily, DNAJB6 & DNAJB8 that we found to be extremely potent suppressors of aggregation initiated by polyglutamine (polyQ) containing proteins in cells. The anti-aggregation activity was dependent on an exclusive C-terminus serine-rich region, the SSF-SST box that is regulated by HDACs and responsible for the formation of large oligomers. Using *in vitro* approaches with purified proteins, we establish that DNAJB6 alone, as a homo-oligomer, directly binds polyQ peptides and hereby greatly delays the "initiation step" of aggregate seeding, surprisingly without needing assistance of Hsp70 family members. The concept of DNAJB6 being working at "initiation" of aggregation process is further strengthened in **Chapter 4**. Here, we first confirm the idea that extracellular polyQ aggregates are internalized by cells and induce non-expanded, non aggregating polyQ proteins to form intracellular aggregates in a prion-like manner. This seeding could not be delayed by intracellular elevation of DNAJB6 expression, suggesting it cannot prevent aggregate propagation.

The next step was to check the efficacy of DNAJB6 to in a mouse model of polyQ disease **(Chapter 5).** Here, we generated transgenic mice with elevated expression of DNAJB6 specifically in the brain. DNAJB6 expression successfully delayed disease onset and increased the lifespan in one of the most severe mouse model of Huntington's disease (R6/2 mouse model). The magnitude of the disease ameliorating effect of DNAJB6 is by far the largest of a single chaperone overexpression in the R6/2 mouse model published and clearly suggest DNAJB6 as target for therapeutic intervention in polyQ diseases.

The data so far in this thesis suggested that members especially within the DNAJB family have potential to ameliorate polyQ disease and we wondered whether they also could be

effective in other protein aggregation diseases. In **Chapter 6**, we did intensive literature survey on role of DNAJs shown so far in the various aggregation diseases. The data showed that indeed various DNAJs may have potential in various aggregation diseases. The data further also support the barcoding concept showing that even within the members of DNAJ family differences exist in specificity and client handling. In addition, the data suggest that an important distinction may have to be made for chaperones that relieve (some of) the consequences of aggregates in a specific disease ("symptomatic relief") and chaperones that delay the initiation of the disease process (i.e. delay protein aggregation).

The findings of the literature survey in **Chapter 6** prompted us to directly compare the different DNAJ members (as done for polyQ proteins) in another aggregation disease **(Chapter 7).** Herefore, we choose to investigate aggregation of the Parkin C289G mutant, which is responsible for juvenile Parkinsonism. Expression of this mutant in cells leads to its aggregation. DNAJB6, as expected, effectively also suppressed Parkin C289G aggregation. Unexpectedly, however, and unlike for suppression of polyQ aggregates, the action of DNAJB6 was independent of its C-terminal SSF-SST but in this case did require cooperation with Hsp70 family members. In fact, we found that up-regulation of all other cytoplasmic DNAJs were equally effective in preventing parkin C289G aggregation and all did so in an Hsp70-dependent manner. This highlights that different aggregation-prone substrates require different chaperone handling consistent with the "barcoding" concept provided in **Chapter 1** and difference between foldable versus non-foldable substrate differences as highlighted in **Chapter 6** (Fig. 2). Moreover, these data suggest that up-regulation of DNAJB6 may not only be beneficial for delaying disease onset in polyQ diseases, but also may have implications in other neurodegenerative protein aggregation disorders.

Chapter 8 summarizes the overall results presented in this thesis with implications and future perspective of the role of chaperones (especially DNAJ family) on protein aggregation diseases. We propose that DNAJs are ideal targets for (disease-specific) therapy not only because they are effective but also because they seem to have little impact on the entire chaperone network, but only redirect (part of) the network to the diseased protein.