Rescue strategies in Drosophila models of neurodegenerative diseases

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Appendices

SUMMARY

In the past decades advances in medicine have led to an extended life span of the general population, which, as a negative consequence, increased the occurrence of age-related neurodegenerative diseases. The necessity to improve the quality of life together with the urge to decrease the economic burden related to patients with neurodegenerative diseases, brings focus to the development of novel treatment strategies for these disorders as the current medical interventions are mostly symptomatic and do not slow down the progression of the diseases.

For our studies of neurodegenerative diseases *Drosophila melanogaster* is used as a model organism due to its unique characteristics that make the fruit fly suitable for large-scale studies and experiments in whole organisms with relatively easily accessible phenotypes. In this thesis, we have proposed three different approaches to treat neurodeneration: 1) a general approach targeting the secondary pathological processes common for several neurodegenerative diseases, 2) a specific approach focusing on the causal element of the disease and 3) a specific approach modulating pathways that could be affected downstream of the disease cause, yet are not directly linked to the origin of the disorder.

In **chapter 2**, our hypothesis about the general approach has been studied in Spinocerebellar ataxia type 3 (SCA3) in *Drosophila*. We tested whether the pathway producing the physiologically active gas hydrogen sulfide (H_2S), which had previously been acclaimed for its therapeutic properties, could modulate the secondary pathological processes in SCA3. It appeared that both the induction of cystathionine-γ-lyase, an H_2S producing enzyme, and supplementation of sodium thiosulfate, an H_2S releasing donor, improved the disease phenotype in flies. To confirm that the effect of this treatment is exhibited on the level of the secondary processes, toxic protein aggregation of the ataxin-3 protein, which characterizes SCA3, was measured upon induction of H_2S synthesis pathway. The results demonstrated that neither the expression, nor the aggregation of the toxic protein was affected by the treatment. On the contrary, the secondary pathological processes, such as increased immune response and oxidative stress, were partially decreased in the treatment conditions. Moreover, we detected that changes in protein persulfidation, which has recently been reported as one of the essential signaling pathways of H_2S, coincided with the rescue of the disease phenotype. This might imply that the effects of inducing the H_2S biosynthesis pathway are exerted via protein persulfidation and that protein persulfidation might play a role in disease progression.

The specific treatment strategy that targets the cause of the disease was applied in the example of Pantothenate kinase-associated neurodegeneration (PKAN) in **chapter 3**. The disease originates from mutations in the enzyme pantothenate kinase that leads to decreased levels of coenzyme A (CoA) in the organism. Therefore, we have attempted to modulate the disease phenotype by replenishing the levels of CoA in the cell. To do so, we tested whether extracellular addition of CoA would increase the levels of intracellular CoA and rescue the PKAN phenotype in the disease model. In this study, we detected a novel source of CoA for the cell: extracellular CoA could be hydrolyzed by ecto-nucleotide-pyrophosphatases to a biologically stable molecule, 4'-phosphopantetheine, which could translocate through the cell membrane by passive diffusion. CoA could be synthesized by the enzyme CoA synthase from 4'-phosphopantetheine after the latter translocated to the cell. The extracellularly supplemented CoA was able to partially rescue the disease phenotype as it had been hypothesized.

One more treatment strategy of neurodegenerative diseases would be to tackle pathways that lead to a particular phenotype downstream of the cause of the disease or the ones that are unrelated to the cause of the disease, yet have common downstream pathways with it. Knowing these pathways would provide a possibility for the specific treatment that is not aimed at the cause of the disease, but aimed at treating the symptoms or aimed at slowing down the progression of the disease. In PKAN, the pleiotropic phenotype of the disease models suggests the presence of various processes and pathways involved in the pathology of the disease. Thus, modulation of candidate genes playing a role in these processes and pathways, outside of the CoA biosynthesis pathway, might have a positive impact on the PKAN phenotype. To identify these possible candidate genes, large-scale genetic and chemical screens should be performed. However, due to the limitations of the existing PKAN models such studies are labor intensive and nearly impossible. In **chapter 4**, we characterized a novel *Drosophila* wing model for PKAN that exhibited three different sub-phenotypes and possessed the characteristics appropriate for large-scale studies. We then validated our model using a compound from the CoA biosynthesis pathway, pantethine, and a candidate pathway from chapter 2, the H_2S biosynthesis pathway. It appeared that both treatments were able to modify two different sub-phenotypes suggesting that the model is applicable for screening studies and that the phenotypes can be modulated independently from one another.

Finally, in **chapter 5** we discussed the results of our studies and their potential application in therapies for patients with neurodegenerative diseases. In this chapter, the questions arising from our data are addressed and possible further steps in this research field are suggested. The main conclusion of this thesis is that the multiple gaps in the treatment strategy of neurodegeneration should be considered from different perspectives – both the general and specific approaches – and the combination of these approaches might be the best solution for the treatment of neurodegenerative diseases.

NEDERLANDSE SAMENVATTING

De vooruitgang die de geneeskunde in de afgelopen decennia heeft geboekt, heeft geleid tot een langere levensduur van de bevolking en, als een negatief gevolg, tot een verhoogde incidentie van leeftijdsgebonden neurodegeneratieve ziekten. Door de noodzaak de levenskwaliteit te verbeteren alsmede door de behoefte om de economische lasten die gepaard gaan met het verzorgen van patiënten met neurodegeneratieve ziekten te verminderen, verschuift het focus naar de ontwikkeling van nieuwe behandelingsstrategieën voor deze aandoeningen. De huidige medische behandelingen zijn immers meestal symptomatisch en derhalve niet genezend.

Wij gebruikten *Drosophila melanogaster* als modelorganisme voor onze studies naar neurodegeneratieve ziekten vanwege zijn unieke kenmerken, die het mogelijk maken om een fruitvlieg voor grootschalige studies en experimenten op het niveau van een intact organisme te gebruiken. In dit proefschrift hebben we drie verschillende benaderingen voor de behandeling van neurodegeneratie voorgesteld: 1) een algemene aanpak gericht op de secundaire pathologische processen , 2) een specifieke aanpak gericht op het causale element van de ziekte en 3) een specifieke aanpak waarmee kandidaten gemodificeerd worden die door de ziekte aangedaan zijn, maar waarvan niet direct duidelijk is dat ze in verband staan met de primaire oorzaak van de ziekte.

In **hoofdstuk 2**, worden onze hypotheses over de algemene aanpak onderzocht met als voorbeeld van spinocerebellaire ataxie type 3 (SCA3) in *Drosophila*. Het fysiologisch actieve gas waterstofsulfide (H_2S) is bekend om zijn therapeutische eigenschappen. In ons onderzoek testten we of kandidaten uit de route van de H_2S productie de secundaire pathologische processen van SCA3 konden moduleren. Het bleek dat zowel de inductie van cystathionine-γ-lyase, een H_2S-producerende enzym, als de toevoeging van natriumthiosulfaat, een H_2S donor, aan het voer van de fruitvliegjes de pathologische fenotypen in fuitvliegjes verbeterden. Om te bevestigen dat het effect van deze behandeling op het niveau van de secundaire processen gebeurt, werd de toxische eiwit aggregatie van het ataxine 3-eiwit, dat SCA3 kenmerkt, na inductie van de H_2S synthese route gemeten. De resultaten toonden aan dat noch de expressie, noch de aggregatie van de toxische eiwitten werden beïnvloed door de behandeling. Integendeel, de secundaire pathologische processen, zoals de verhoogde immuunrespons en oxidatieve stress, werden gedeeltelijk verminderd door de behandeling. Bovendien ontdekten we dat veranderingen in de eiwit persulfidatie, dat recent is gerapporteerd als één van de essentiële signaalroutes van H_2S, geassocieerd was met verbetering van het fenotype. Dit zou kunnen betekenen dat het effect van het induceren van de H_2S biosyntheseroute via het eiwit persulfidatie wordt uitgevoerd en dat eiwit persulfidatie een rol in de ziekteprogressie kan spelen.

De specifieke behandelingsstrategie die op de oorzaak van de ziekte gericht is wordt in **hoofdstuk 3** uitgelicht. De neurodegeneratieve ziekte die wij hebben bestudeerd was pantothenate kinase-geassocieerde neurodegeneratie (PKAN). Deze ziekte wordt veroorzaakt door mutaties in het enzym pantothenate kinase, die leiden tot verminderde niveaus van co-enzym A (CoA) in het organisme. Onze hypothese was daarom dat we het fenotype konden verbeteren door middel van aanvulling van de niveaus van CoA in de cel. Om dit te doen, hebben we getest of extracellulaire toevoeging van CoA de niveaus van intracellulair CoA zou bijvullen en vervolgens het PKAN fenotype zou redden. In deze studie hebben we een nieuwe bron van CoA voor de cel uitgevonden: extracellulaire CoA kan worden gehydrolyseerd door ecto-nucleotide-pyrofosfatasen tot een biologisch stabiel molecuul, 4'-phosphopantetheine, dat door het celmembraan middels passieve diffusie kan transloceren. CoA kan worden gesynthetiseerd uit 4'-phosphopantetheine door het enzym CoA-synthase nadat deze translocatie naar de cel plaats heeft gevonden. Het extracellulair aangevulde CoA kon het ziekte fenotype gedeeltelijk redden zoals door ons was verondersteld.

Een andere behandelingsstrategie van neurodegeneratieve ziekten richt zich op routes die specifiek bij een bepaalde ziekte zijn aangedaan en die een deel van de symptomen kunnen verklaren. Kennis van deze routes zou een specifieke behandeling mogelijk maken die niet gericht is op de oorzaak van de ziekte, maar wel op het behandelen van de symptomen of ter vertraging van de progressie van de ziekte. PKAN heeft een zeer pleiotroop fenotype en dit suggereert de aanwezigheid van verschillende processen en routes die betrokken zijn bij de pathologie van de ziekte. Modulatie van de kandidaatgenen, die een rol in deze processen en routes buiten de CoA biosyntheseroute spelen, kan dus een positieve invloed op het PKAN fenotype hebben. Om deze mogelijke kandidaatgenen te identificeren, moeten grootschalige genetische en chemische screens worden uitgevoerd. Vanwege de beperkingen van de bestaande modellen van PKAN zijn dergelijke studies arbeidsintensief en bijna onmogelijk. In **hoofdstuk 4**, hebben we een nieuw *Drosophila* vleugelmodel voor PKAN ontworpen dat drie verschillende sub-fenotypes toonde en geschikt was voor grootschalige studies. Vervolgens hebben we ons model gevalideerd door een stof uit de CoA-biosyntheseroute, pantethine, toe te voegen, en de kandidaatroute uit hoofdstuk 2, de H_2S biosyntheseroute te activeren. Het bleek dat beide behandelingen twee verschillende sub-fenotypen konden modificeren. Deze resultaten suggereren dat het model voor screeningsstudies gebruikt kan worden en dat de fenotypen onafhankelijk van elkaar gemoduleerd kunnen worden.

Tenslotte bespreken we in **hoofdstuk 5** de resultaten van ons onderzoek en de mogelijke toepassingen op de therapie van patiënten met neurodegeneratieve ziekten. In dit hoofdstuk worden de vragen die voortkomen uit onze data aangepakt en de mogelijke verdere stappen in dit onderzoeksveld voorgesteld. De belangrijkste conclusie van dit proefschrift is dat de behandelingsstrategie van neurodegeneratie vanuit verschillende perspectieven moet worden beschouwd - zowel de algemene als de specifieke aanpak - en dat de combinatie van deze benaderingen wellicht de beste oplossing is voor de behandeling van neurodegeneratieve ziekten.

LIST OF PUBLICATIONS

Baratashvili M, Snijder PM, Leuvenink HGD, Kuijpers L, Huitema S, Schaap O, Giepmans BNG, Kuipers J, Miljkovic JLj, Mitrovic A, Bos EM, Csaba Szabó, Kampinga HH, Dijkers PF, den Dunnen WFA, Filipovic MR, van Goor H, Sibon OCM
Overexpression of cystathionine γ-lyase suppresses detrimental effects of spinocerebellar ataxia type 3
Mol Med 2015; 21: 758-768.

Srinivasan B, **Baratashvili M**, van der Zwaag M, Kanon B, Colombelli C, Lambrechts RA, Schaap O, Nollen EAA, Podgoršek A, Kosec G, Petković H, Hayflick S, Tiranti V, Reijngoud DJ, Grzeschik NA, Sibon OCM
Extracellular 4'-phosphopantetheine is a source for intracellular Coenzyme A synthesis
Nat Chem Biol 2015; 11: 784-92.

Crippa V, Cicardi ME, Seguin SJ, Ganassi M, Bigi I, Diacci C, Zelotti E, **Baratashvili M**, Gregory JM, Dobson CM, Cereda C, Pandey UB, Poletti A, Carra S
HspB8 counteracts truncated TDP43 accumulation protecting against TDP43-mediated toxicity in cells and in Drosophila melanogaster
Hum Mol Genet (under revision)

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