Project title: Tau and TDP-43 at cross-roads in neurodegeneration: Spatiotemporal interaction study in neurons

Supervision: Oxford Supervisors: Dr Martina Hallegger and Dr. Steph Fowler (Primary supervisors), Dr. Avigail Taylor (Oxford co-supervisor) and Dr. John Todd (Oxford co-supervisor); GSK supervisor TBD

Summary:

The incidence of TDP-43 co-pathology in neurodegenerative disorders wherein tau is the predominant driver of disease is between 20-60%. Indeed, up to 57% of Alzheimer's Disease (AD) cases present with comorbid limbic predominant age-related TDP-43 encephalopathy neuropathological changes (LATE-NC), and are associated with more severe clinical outcomes compared to AD cases lacking TDP-43[1]. Even more strikingly, all cases of fronto-temporal lobar dementia (FTLD) with TDP as the driving pathology (FTLD-TDP) display concurrent tau pathologies of some type (ARTAG (42%), PART (36%), and AGD (22%))[2].

There is growing evidence that the presence of TDP-43 exacerbates tau pathology via a nuanced and complex feedback mechanism. In both murine and human iPSC models of TDP-43 loss of function, tau becomes more sensitive to caspase-3-dependent cleavage, dissociation from microtubules, and subsequent assembly[3]. However, instead of promoting the development of fibrillar tau pathology, the presence of TDP-43 in the cytoplasm appears to drive the formation of small, oligomeric tau and mixed tau/TDP-43 species[4]. Paradoxically, these mixed tau/TDP-43 co-assemblies (either produced in-vitro, or isolated from patient brain material) have reduced tau seeding activity in biosensor assays compared to tau-only assemblies. Whilst less effective at templating pure tau fibrils, these mixed oligomers are particularly neurotoxic, and are also more highly potent at seeding TDP-43 assembly. Similar to diseases wherein tau is the driving pathology, the presence of tau pathology in TDP-43-predominant neurodegenerative diseases like FTLD-TDP43 or ALS also accelerates TDP-43 assembly by promoting its mis-localisation from the nucleus[5].

Despite mounting neuropathological and molecular evidence that misfolded tau and TDP-43 pathologies co-exist, our understanding of the spatial context, timing, and relationship between these two proteopathic proteins is not well-defined. We are interested in dissecting where, when, and how tau and TDP-43 interact in human neurons using the 4R i3Neuron lines recently developed in the Fowler lab that display assembly and pathological tau hyperphosphorylation (AT8+ signal) when exposed to exogenous tau seeds. The objective of the project is to further our understanding of the complex interplay between these two concomitant pathologies to support successful multi-target therapeutic development in AD and FTD. We hypothesize that tau and TDP-43 could interact at a variety of subcellular sites including the nucleus, within cytoplasmic stress granules, and possibly at or within mitochondrial organelles. The spatial organisation of co-aggregates will likely change as pathology proceeds, and interactions may depend on the assembly states of both proteins. We anticipate that tau-TDP-43 interactions could be mediated both by direct protein-protein interaction and/or the influence of specific RNA(s) that may serve as tethers for co-condensates.

Project Aims:

1) To determine where and when within neurons tau and TDP-43 begin to interact, we will use a highly-resolved cellular fractionation protocol to spatially map subcellular sites of tau and TDP-43 co-localisation across the development of tau pathology in i3Neurons. Tau and TDP-43 protein:protein interactions will be assessed and validated by co-immunoprecipitation and super-resolution microscopy in intact neurons [Output: spatial proteomics/interactomics dataset]

2) To determine if RNA molecules mediate the interaction of tau and TDP-43 in relevant subcellular fractions identified in Aim 1, we will identify RNAs using SPRITE (Split-Pool Recognition of Interactions by Tag Extension), and will map their interaction with respective protein interactors (RBPs; RNA binding proteins) using SPIDR (Split-and-Pool Identification of RBP targets), a highly multiplexed method for profiling the global RNA binding sites of hundreds of RBPs in a single experiment. [Output: spatial transcriptomics dataset]

3) To develop a model for how tau and TDP-43 pathologies synergise in neurons, we will integrate the spatial proteomics and transcriptomics data from Aims 1 and 2. Prioritised models will be tested and validated in intact neurons and post-mortem human brain tissue.

Potential applications and benefits: The current project will generate new knowledge surrounding the location and nature of tau/TDP-43 co-assemblies. The spatial proteomics and transcriptomics outputs will provide a rich base for identifying both protein and RNA co-factors of tau-TDP-43 co-assemblies, which could inform new strategies for reducing the interaction of these two proteopathic proteins in a wide variety of neurodegenerative conditions. No ethical approvals for data sharing with industry partners are known.

Alignment with therapeutic area and key scientific theme(s):

Therapeutic area: Alzheimer's Disease and ALS
Theme: Role of the CNS and peripheral immune system in neurodegeneration.

Project delivery:

In the first year the student will be trained to differentiate 4R i3Neurons that display assembly and pathological tau hyperphosphorylation. They will conduct a highly-resolved cellular fractionation (based on the Lilley Lab's LOPIT workflows), following the Fowler lab's established adaptation of these methods to cultured i3Neurons. The data set resulting from Aim 1 will also be mined for other RNA binding proteins (RBPs) that indicate localisation changes associated with tau seeding. In the second year, these fractions will be used for sophisticated transcriptomics techniques including SPRITE to look for altered RNA localisation. The RNA-interactome of each fraction will be mapped through multiplexed immunoprecipitations against TDP-43, tau, and newly discovered RBPs to discover their co-assembly on RNAs using SPIDR. Year three will be dedicated to data analysis and integration, followed by manuscript preparation and thesis writing. Martina Hallegger, Steph Fowler, and Avigail Taylor (IMCM Technical Lead in Bioinformatics) will provide the overall and day-to-day supervision on the data analysis. The student will be embedded in the IMCM bioinformatics team at stages of intense bioinformatics training. Working in the cloud-based Oxford-GSK trusted research environment (TRE), the student will use pre-established and pre-tested bioinformatics pipelines. SPRITE requires standard small RNAseq data analysis and R-based transcript colocalization analysis. SPIDR analysis will be based on published pipelines (https://github.com/mjlab-Columbia/spidr-paper-pipeline). The project ties in with the ongoing IMCM AD project on brain multi-omics co-lead by Becky Carlyle and Laura Parkkinen. Proteins and RNAs identified in our study will be collaboratively analysed in post-mortem tissues.

Research environment:

Our supervisory team is well-placed to address the crosstalk between TDP-43 and tau pathology. The Fowler Lab has extensive expertise in modelling tau aggregation in cells and analysis of highly-resolved cellular fractionation and proteomics thereof. Additionally, they also have developed unique mutant tau i3Neuron models that display assembly and pathological tau hyperphosphorylation. The Hallegger lab has all the required transcriptomics expertise and has a well-established track-record for setting up novel transcriptomics techniques. Martina Hallegger is an expert in TDP-43 RNA interaction, TDP-43 granule formation, and the iCLIP method (highly complementary to both SPRITE and SPIDR techniques). Together with the bioinformatics expertise of the IMCM computational team under the lead of Avigail Taylor, we will develop technical platforms to address key unanswered questions related to the spatial transcriptomic signatures of tau and TDP-43 co-pathologies, with the aim of identifying and validating early potential drug targets and biomarkers to predict disease progression.

References:

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