HCB-01: Biological relevance of a spliced variant of hnRNP A1

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Background:
The development of an effective treatment for ALS is urgent and is expected to be informed by a better understanding of the molecular mechanisms underlying ALS. TDP-43 mislocalization from the nucleus to the cytoplasm is observed in the majority of ALS cases, in nearly half of FTD cases as well as in other neurodegenerative diseases. We have discovered that nuclear TDP-43 depletion drives the formation of an alternatively spliced variant of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), termed hnRNP A1B. hnRNP A1 is a major player in RNA metabolism and one of the most abundant proteins in motor neurons. It is mutated in rare cases of familial ALS and these disease-associated mutations drive aggregation of hnRNP A1. hnRNP A1 is well studied, but surprisingly little is known about this spliced variant. hnRNP A1B has an elongated prion-like domain (PrLD) and we have previously shown that this is associated with increased aggregation propensity and cell death (Deshaies et al., Brain, 2018).

We hypothesize that the extended PrLD domain of hnRNP A1B facilitates unique protein-protein interactions compared to hnRNP A1 and thus contributes to distinct cellular functions.

1. Evaluate the spatiotemporal expression of hnRNP A1B in development and aging.
2. Identify hnRNP A1B neuronal functions via interrogation of its interactome.

Methods:
To uniquely identify hnRNP A1B, we have generated a polyclonal antibody targeting the residues encoded by the alternatively spliced residues in exon 7B exon and thus are unique to hnRNP A1B. We have used this specific antibody to evaluate its spatial temporal expression in healthy mice. We have also used immunoprecipitation coupled to mass spectrometry to identify the hnRNP A1B proteome in mouse spinal cords.

Results:
We have determined that hnRNP A1B is predominantly expressed in the central nervous system (CNS) of mice compared to hnRNP A1. Its global expression is high and ubiquitous in young animals and becomes gradually restricted to a subset of neurons in the spinal cord during aging. While both hnRNP A1 isoforms demonstrate robust nuclear labeling, compared to hnRNP A1, hnRNP A1B also exhibits a cytoplasmic distribution.
We also report on novel hnRNP A1B protein-protein interactions that have not been previously associated with hnRNP A1. These novel interactions suggest involvement of hnRNP A1B in RNA metabolism and cellular transport. Functional experiments to interrogate these pathways are underway.

Discussion:
Persistent hnRNP A1B expression in neurons of the CNS supports the idea that it plays a crucial biological function in these cells. The cytoplasmic distribution of hnRNP A1B and its proteome indicates a potential novel function for hnRNP A1B in RNA transport, a crucial pathway for neuronal development and function.

Acknowledgments:
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HCB-02: C9ORF72/SMCR8/WDR41 complex as a GTPase activating protein (GAP) for small Rho GTPases

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C9ORF72/SMCR8/WDR41 complex as a GTPase activating protein (GAP) for small Rho GTPases

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease that affects motor neurons ultimately resulting in respiratory failure[1]. C9ORF72 (non-coding hexanucleotide repeat expansion), the most commonly mutated protein linked to ALS[2],[3], forms a complex with SMCR8 and WDR41. The complex has been shown to be involved in numerous functions revolving cellular trafficking making it difficult to determine the exact function of the complex.

We have discovered that C9ORF72 is expressed at high levels in macrophages and localizes to phagosomes and lysosomes[3]. Macrophages take up debris into early phagosomes facilitated by the rearrangement of actin coat[4] regulated by Rho family GTPases[5]. Disassembly of actin coat is required for fusion of phagosomes with lysosomes, which is regulated by Rho GAPs[6], and this step overlaps temporally with the localization of C9ORF72 to phagosomes[3], suggesting that the complex may act as a GAP for small Rho GTPases.

Our mass spectrometry analysis of the affinity selected proteins (purified complex coupled to beads incubated with macrophages lysate) revealed several small GTPases including CDC42, RhoG, Rac2, Rho F and Rho A taking our hypothesis one step further. All the candidates will be tested as binding partners using bioluminescence-based GTPase assay. We will also analyze the effect of the mutated complex on phagocytic activity of macrophages. The identification of the substrate will help identify the function of the complex and hence the pathways to be targeted for therapeutic purposes.

References:

1. Al-Chalabi, A., Hardiman, O., Kiernan, M. C., Chiò, A., Rix-Brooks, B., & Berg, L. H. (2016). Amyotrophic lateral sclerosis: Moving towards a new classification system. The Lancet Neurology, 15(11), 1182-1194. doi:10.1016/s1474-4422(16)30199-5
2. Dejesus-Hernandez, M., Mackenzie, I., Boeve, B., Boxer, A., Baker, M., Rutherford, N., . . . Rademakers, R. (2011). Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. Neuron, 72(2), 245-256. doi:10.1016/j.neuron.2011.09.011
3. Laflamme, C., Mckeever, P. M., Kumar, R., Schwartz, J., Kolahdouzan, M., Chen, C. X., . . . Mcpherson, P. S. (2019). Implementation of an antibody characterization procedure and application to the major ALS/FTD disease gene C9ORF72. ELife, 8. doi:10.7554/elife.48363
4. Chimini, G., & Chavrier, P. (2000). Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nature Cell Biology, 2(10). doi:10.1038/35036454
5. Hoppe, A. D., & Swanson, J. A. (2004). Cdc42, Rac1, and Rac2 Display Distinct Patterns of Activation during Phagocytosis. Molecular Biology of the Cell, 15(8), 3509-3519. doi:10.1091/mbc.e03-11-0847
6. Schlam, D., Bagshaw, R. D., Freeman, S. A., Collins, R. F., Pawson, T., Fairn, G. D., & Grinstein, S. (2015). Phosphoinositide 3-kinase enables phagocytosis of large particles by terminating actin assembly through Rac/Cdc42 GTPase-activating proteins. Nature Communications, 6(1). doi:10.1038/ncomms9623

HCB-03: Changes in pathological phenotype of C9orf72 ALS iPSC-derived lines after treatment with Morpholino oligomers

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Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive degeneration of motor neurons (MNs). GGGGCC repeat expansions in C9ORF72 gene are the most common identified genetic cause, and even if their pathogenic processes are still unknown, many possible mechanisms have been proposed, including loss of function of the C9Orf72 protein, gain of function from accumulation of RNA foci and sequestration of RNA binding proteins (RBPs), and toxicity caused by dipeptide repeats proteins (DPRs) produced by repeat-associated non-ATG (RAN) translation. One promising and reliable method to understand C9-ALS pathogenesis is represented by patient-specific induced pluripotent stem cells (iPSC)-derived lines and iPSC-derived MNs. Our therapeutic approaches include the use of antisense oligonucleotides (ASOs) designed to bind complementary mRNA and interfere with specific biological processes. In our laboratory, two different ASOs with Morpholino chemistry have been designed: against the C9ORF72 expansion motif and against the whole C9ORF72 gene; our aim is to characterize the pathological phenotype of the C9-ALS iPSC-derived lines and evaluate the therapeutic effect of ASOs administration on specific pathological markers. We reprogrammed iPSCs from C9-ALS patients and controls and differentiated them into MNs using a 14-days protocol. We investigated the phenotype of the C9-ALS lines compared to controls, evaluating cells survival, pluripotency and motor neuronal markers, TDP43 inclusion presence, R-loops formation, STMN2 expression, defects in axonal elongation and nucleolar disfunctions. Next step was transfecting ALS-MNs with different Morpholinos and evaluating modification of the previously mentioned pathological markers. Interestingly, we identified in C9-ALS iPSC-derived lines pathological features such as accumulation DNA damage, R-loops increase, and minor axonal elongation, with decreased levels of Nfh, Stmn1 and Sept7 genes. After Morpholino treatments, we observed that ASO therapy could partially rescue the pathological phenotype. Our results suggest that patient specific iPSCs and iPSC-derived MNs are a valuable tool to deepen the knowledge of C9ORF72 pathogenic mechanisms, and that Morpholino-mediated approaches represent a very promising therapeutic strategy that needs to be further validated.

HCB-04: Characterising TDP-43 aggregation in ALS at the nanometre-length scale.

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Background:
TAR DNA-binding protein 43 (TDP-43) has been identified as the main constituent of proteinaceous inclusion bodies found in ALS, and is a key protein involved in other neurodegenerative diseases [1, 2]. Indeed, in over 95% of ALS cases, TDP-43 mislocalises from its typical cellular location in the nucleus to the cytosol, where it forms toxic inclusions. However, studying the aggregation of TDP-43 has proven to be exceedingly difficult. In vitro studies of the full-length protein are hampered by its propensity to precipitate during the purification process, while in cellulo and in vivo work is complicated by the current lack of specific and precise labelling methods for TDP-43.

Objectives:
We aim to develop imaging tools and techniques that allow for an unprecedented level of detail in TDP-43 aggregate imaging. Furthermore, we want to apply these to the imaging of TDP-43 aggregates in highly patient-relevant samples throughout ALS.

Methods:
We have developed a novel aptamer-based imaging probe compatible with super-resolution imaging approaches to observe TDP-43 aggregates at the single-molecule level. Our approach enables the structural characterisation of TDP-43 aggregates at the nanometer length scale. We can utilise these tools to study various in vitro constructs of TDP-43, and image aggregates in a wide variety of patient-relevant samples, including patient-derived motor neurons and glial cells, and post-mortem brain tissue.

Results:
Using the aptamer imaging approach, we have been able to image TDP-43 aggregation in super-resolution for the first time, giving an unprecedented level of detail. We have made significant progress in the imaging of post mortem patient tissue, where we are characterizing aggregates on a much more sensitive scale than was previously possible. Therefore, revealing details of aggregation behavior, aggregate sizes and distributions in human ALS samples.
Current work also focuses on imaging TDP-43 inclusions in a range of cell models, including C9ORF72 repeat expansion motor neurons.

Discussion:
It is hoped that these methods will not only allow the detailed study of TDP-43's mislocalisation and aggregation, but also show how TDP-43 aggregates induce toxicity and how they interact with and disrupt cellular processes. The mentioned probes also show potential in the search for diagnostically relevant biomarkers in CSF and as tools to ameliorate disease pathology by manipulating the misfolding and location of TDP-43.

[1] M. Neumann et al., "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis," Science, vol. 314, no. 5796, pp. 130-3, Oct 6, 2006.
[2] P. T. Nelson et al., "Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report," Brain, Apr 30, 2019.

HCB-05: Compositional analysis of physiological stress granules and ALS-linked stress granule-like structures reveals factors and cellular pathways dysregulated by mutant FUS under stress

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Formation of cytoplasmic RNA-protein structures called stress granules (SGs) is a highly conserved cellular response to stress. Abnormal metabolism of SGs may contribute to the pathogenesis of a number of (neuro)degenerative diseases such as amyotrophic lateral sclerosis (ALS). Many SG proteins are affected by mutations causative of these conditions, including fused in sarcoma (FUS). Mutant FUS isoforms have high affinity to SGs and also spontaneously form de novo cytoplasmic RNA granules. Mutant FUS-containing SG-like assemblies (mFAs), often called “pathological SGs”, are proposed to play a role in ALS-FUS pathogenesis. However, the structural and functional differences between mFAs and physiological SGs remain largely unknown therefore it is unclear whether and how mFAs affect cellular stress response. Here we used affinity purification to characterise the protein and RNA composition of normal SGs and mFAs purified from stressed cells. Comparison of the SG and mFA proteomes revealed that proteasome subunits and certain nucleocytoplasmic transport factors are depleted, whereas translation elongation, mRNA surveillance and splicing factors as well as mitochondrial proteins are more enriched in mFAs as compared to SGs. Validation experiments for a hit from our analysis, a splicing factor hnRNPA3, confirmed its RNA-dependent sequestration into mFAs in cells and into pathological FUS inclusions in a transgenic ALS-FUS model that may lead to its loss of function. Furthermore, silencing of the Drosophila hnRNPA3 ortholog dramatically enhanced FUS toxicity in a fly ALS-FUS model. Comparative transcriptomic analysis of SGs and mFAs revealed that mFAs recruit a significantly less diverse spectrum of RNAs, including reduced recruitment of transcripts involved in protein translation, DNA damage response, microtubule organisation and apoptotic signalling. However mFAs but not SGs sequester certain transcripts involved in stress signalling cascades. Our study establishes molecular differences between physiological SGs and mFAs and identifies the spectrum of proteins, RNAs and respective cellular pathways affected by mFAs in stressed cells. In conclusion, we show that mFAs are compositionally distinct from SGs and that they cannot fulfil SG function while gaining novel, unwanted functions in cellular stress response. Results of our study support a pathogenic role for stress-induced cytoplasmic FUS assemblies in ALS-FUS.

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HCB-07: Dipeptide repeat proteins interactome identification by proximity-dependent labeling with BioID2

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Background:
Hexanucleotide GGGGCC repeat expansion within C9orf72 gene is the most common genetic cause of ALS and FTLD. One of the proposed mechanisms of GGGGCC repeats is their translation to produce unnatural dipeptide repeat (DPR) proteins that accumulate in aggregates and contribute to the pathology. There are 5 different DPRs poly(GA), poly(GR), poly(PR), poly(PA), poly(GP) which are neurotoxic in vitro[1]. Poly(GA) is the most hydrophobic and forms distinct perinuclear aggregates, whereas the highly charged poly(PR) and poly(GR) tend to accumulate in nucleoli and cytoplasm respectively. They all have been previously shown to interact with ribosomes, stress granules and low-complexity proteins[2].

Objectives:
To elucidate the mechanism and function of DPRs we analyzed the interactome of all five DPR proteins, consisting of 125 repeats.

Methods:
We overexpressed all five DPR proteins with 125 repeats in HEK293T cells. Using BioID2 proximity labeling we made pull-down purification of the biotinylated protein candidates[3] and performed LC-MS proteomic analysis. After bioinformatic analysis selected protein candidates were validated by immunoblotting and immunocolocalization.

Results:
We identified 140 interacting partners for poly (GR), 130 for poly(GA), 138 for poly(PR), 62 for poly(PA), and 38 for polyGP. Gene ontology enrichment analysis of proteomic data identified interaction candidates involved in protein translation, signal transduction pathways, protein catabolic processes, amide metabolic processes and RNA-binding. Finally, we proceeded with determination of mechanism of poly(GA) – VCP interaction, our most potent interacting partners.

Discussion:
In this study we show the interactome of all five DPR proteins consisting of 125 repeats and overlapping of these interacting partners. Furthermore, we confirmed the interaction of VCP protein and poly(GA) protein in cells and in brain tissue of ALS patients and proposed how this interaction contribute to the pathogenesis.

Reference:
[1] X. Wen, W. Tan, T. Westergard et al., Neuron 84 (2014) 1213-1225
[2] H. Hartmann, D. Hornburg, M. Czuppa et al., Life Sci Alliance 1 (2018) e201800070
[3] D.I. Kim, S.C. Jensen, K. A. Noble et al., Mol. Biol. Cell 27 (2016) 1188–96

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HCB-08: DRP1 and tau induce mitochondrial fragmentation in Amyotrophic Lateral Sclerosis

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Background:
Previous studies have demonstrated that mitochondrial dysfunction is an early pathogenetic event in amyotrophic lateral sclerosis (ALS). Accordingly, deficits in bioenergetics and mitochondrial function have been reported in ALS patient samples as well as in animal and cellular models of disease. Studies in Alzheimer's disease post-mortem brain and animal models link alterations in mitochondrial function to interactions between hyperphosphorylated tau and dynamin-related protein 1 (DRP1), the GTPase involved in mitochondrial fission. Here, we sought to verify whether the accumulation of DRP1 may lead to mitochondrial fragmentation and dysfunction in ALS.

Methods:
Synaptoneurosome fractionations (SNs) were prepared from a large cohort of post-mortem motor cortex (mCTX) from ALS patients and controls. Western blots were used to assess the levels of proteins involved in mitochondrial dynamics in SNs. Electron microscopy was used to assess mitochondrial length in mCTX. Interactions between tau and DRP1 were measured using co-immunoprecipitation (Co-IP). SH-SY5Y cells were used to assess mitochondrial length, volume, and networks following treatment with tau, ALS or control SNs in the absence or presence of DRP1 silencing (siDRP1) and a selective tau degrader (QC-01-175). Genetic variants were identified using the ALS Knowledge Portal (ALSKP) and Project MinE data browser.

Results:
Our results demonstrated a significant increase in DRP1 and its active phosphorylated isoform (pDRP1-S616) in ALS SNs. Importantly, increases in DRP1 were independent of sex, region of onset and genotype. Interestingly, co-IP studies demonstrated that DRP1 interacts with pTau-S396 in ALS mCTX, suggesting that tau hyperphosphorylation may contribute to mitochondrial dysfunction in ALS. Moreover, treatment of SH-SY5Y cells with ALS SNs, enriched in both pTau and DRP1, significantly increased mitochondrial fission by reducing mitochondrial length and volume. Importantly, knocking down DRP1 using a specific siRNA or reducing tau levels using QC-01-175, significantly mitigated alterations in mitochondrial length and volume induced by ALS SNs. Lastly, we identified specific genetic variants in DNM1L in ALS cases by assessing the ALSKP and Project MinE.

Discussion:
Together, our findings suggest that increases in DRP1 and pTau may cause mitochondrial fragmentation in ALS, leading to an unfavorable energic state and contributing to motor neuron loss. Importantly, targeting this molecular pathway may provide a novel therapeutic strategy for ALS.

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HCB-09: Dysregulated circRNAs in ALS colocalize with RNA granules upon stress

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Introduction:
The disruption of normal RNA transport and metabolism has been proposed to be the functional consequence of genetic mutations linked to ALS. Several genetic mutations associated with ALS exist in genes encoding RNA-binding proteins (RBPs), which are involved in gene transcription, mRNA splicing, RNA stability, microRNA (miRNA) processing, RNA splicing, and transport. Given this, and the observations of that coding and non-coding RNA expression and RNA stability is altered, ALS is increasingly considered to be a disorder of altered RNA metabolism. Complex processes of RNA metabolism occur in membrane-less ribonucleoprotein (RNP) granules. Several studies have explored the role of RNA granules in ALS (1). Long coding and non-coding RNAs play crucial nucleation and structural roles in RNA granules (2). A less studied group of non-coding RNAs are covalently closed circular RNAs (circRNAs) which are conserved across mammals. CircRNAs are highly expressed in mammalian nervous system, and their expression levels are dynamically modulated in neurons (3).

Objectives:
We investigated in vitro the effect of osmotic stress on the localization and expression of a selection of circRNAs whose expression is dysregulated in ALS (hsa_circANXA1_001, hsas_circDNM1_004, hsa_circVIM_005 and hsa_circVIM_011).

Methods:
We first identified a pool of circRNAs whose expression was altered in human spinal cord tissues of sporadic ALS patients using whole transcriptome RNA-seq (Illumina, Partek/GSA analysis). Using a combination of fluorescence in situ hybridization (FISH) using LNA probes against a group of dysregulated circRNAs and immunofluorescence (IF) using markers for different RNA granules, we examined the subcellular localization of these circRNAs in HEK293T cells under osmotic stress (400 mM sorbitol, 4h) and non-stress conditions. Subcellular localization was assessed using a Leica SP8 MP laser scanning confocal microscope and quantitation performed using Leica Confocal Software (LASX) and FIJI/ImageJ software.

Results:
Under basal conditions, we observed a range of either nuclear or cytosolic predominant localization, or both, for the 4 circRNAs studied. Following stress, we observed that a subgroup of the circRNAs colocalized with markers of RNA granules, including TIA-1 (stress granules), DCP-1 (p-bodies), and staufen (transport granules). In preliminary studies, we also observed cytosolic colocalization with paraspeckle proteins under stress conditions for circDNM1. Studies are in progress to determine the extent to which circRNA expression levels are altered under stress condition.
Discussion: We have observed that circRNAs are present in RNA granules under a stress condition. This finding is consistent with the hypothesis that circRNAs can participate in the formation of RNA granules under pathological conditions such as those observed in ALS.

References:
1. Strong MJ. Journal of the neurological sciences. 2010 ;288(1-2):1-2.
2. Naganuma Tet al. RNA biology. 2013 ;10(3):456-61.
3. Rybak-Wolf A et al. Molecular cell. 2015;58(5):870-85.

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HCB-10: Establishing a novel in vitro model of amyotrophic lateral sclerosis by co-culturing induced pluripotent stem cell-derived motor neurons and microglia

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Background:
A growing body of evidence supports a role for neuroinflammation in amyotrophic lateral sclerosis (ALS) pathophysiology, with microglia particularly implicated. However, their specific role, harmful or protective, at different stages of the disease course and in the context of different ALS mutations is unresolved (1). To date, studies on microglia have been largely restricted to animal models, and there remains a need for more authentic disease models using human cells. In our previous work (2), human induced pluripotent stem cell (iPSC)-derived microglia co-cultured with iPSC-derived cortical neurons expressed relevant microglia markers, displayed dynamic ramifications, and were functionally active with respect to phagocytosis and secretion.

Objectives:
This project aims to co-culture iPSC-derived microglia with iPSC-derived motor neurons (MNs) to establish a novel in vitro model of neuroinflammation in ALS.

Methods:
We differentiated iPSC-derived microglia and MN precursors from three healthy controls as described previously (3-5) and optimised the culture medium in order to establish compatibility between both cell types. We then combined both microglia and MN precursors in co-culture and maintained cultures to allow maturation of both cell types. Co-cultures were analysed for the maturity and identity of both MNs and microglia.

Results:
MNs grown in co-culture medium alone and in co-culture with iPSC-derived microglia retained neuronal morphology and showed similar expression of the neuronal and MN markers TUJ1, ISLET-1, and ChAT compared to MNs cultured in MN medium. Moreover, co-cultured MNs displayed clear spontaneous activity in calcium imaging. iPSC-derived microglia in co-culture were IBA1-positive, made direct contact with MNs and their neurites, and showed ramifications with highly dynamic remodelling of primary and secondary branches. Furthermore, co-cultured iPSC-derived microglia were phagocytically competent.

Discussion and conclusion:
These findings provide preliminary evidence for the functionality of our co-culture model. We are now evaluating the expression of key microglia markers and the secretory profile in co-culture. This co-culture model will further our understanding of the functional role of microglia in ALS.

References:
1. Geloso MC, et al. Front Aging Neurosci. 2017;9:242.
2. Haenseler W, et al. Stem Cell Reports. 2017;8(6):1727-42.
3. van Wilgenburg B, et al. PLoS One. 2013;8(8):e71098.
4. Dafinca R, et al. Stem Cell Reports. 2020;14(5):892-908.
5. Dafinca R, et al. Stem Cells. 2016;34(8):2063-78.

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HCB-11: Exploiting iPSC-derived motor neurons and exosomes for the development of a miRNA-based therapeutic strategy in ALS

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by a progressive degeneration of motor neurons (MNs). Most of the cases are sporadic (sALS) while familial ALS (fALS) occurs only in 10% of all subjects. A dysregulation of microRNA (miRNA) expression in ALS has been already described, although downstream pathological events associated with MN degeneration have not been clarified yet. miRNAs are highly expressed in central nervous system thus they may play important roles in the etiology or progression of neurodegenerative diseases such as ALS.
In this study, we aimed at investigating whether alteration of miRNA expression patterns in ALS-MNs may represent a common molecular feature among the different forms of the disease. We performed differential expression profile analysis of miRNAs isolated from iPSC-derived MNs of ALS patients (SOD1, TARDBP and C9ORF72) and healthy subjects. We identified a small group of downregulated miRNAs in ALS-MNs. Interestingly, a dysregulation of the same subset of miRNAs has been detected in exosomes released from the same ALS-MN cultures. Since bioinformatic analysis showed that these miRNAs regulate several pathways related to MN degeneration, we investigated their potential as disease biomarkers assessing their expression level in cerebrospinal fluid (CSF) of ALS patients. We confirmed a different expression pattern of these miRNAs in CSF isolated from ALS subjects suggesting their potential clinical relevance.
Taken together our results demonstrate that the neurodegenerative phenotype in ALS can be associated with a dysregulation of miRNAs involved in the control of disease-relevant molecular pathways. The possibility of tuning entire gene networks with a specific subset of miRNAs may provide significant insights on the development of effective new miRNA-based therapies and could be useful as disease biomarkers.

HCB-12: Exploring the the Neuroinflammatory Response of the Motor Regions in Human Motor Neurone Disease.

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Purpose:
While predominantly affecting motor neurones, it is clear that other cells contribute to pathogenesis of Motor Neurone Disease (MND). Microglia, the primary immune cells of the central nervous system are highly involved, and their activation has been found to correlate with a number of features of disease. However, the function of microglia and how microglia contribute to human MND is not currently known. We aim to elucidate the role of immunity in sporadic MND by examining gene expression in post mortem tissue.

Methods:
Transcriptional analysis was performed using the nanoString Neuroinflammation panel of 770 genes. RNA was extracted from frozen cervical spinal cord anterior horn and motor cortex from MND patients (16 cases per location) and controls (8 cases per location).

Results:
In controls and MND cases, 221 and 330 genes, respectively, were differentially expressed between anterior horn and motor cortex, indicating the spinal cord may be a more immune environment compared to the motor cortex both in disease and homeostatic conditions.

In MND cases compared to controls, 128 genes were differentially expressed in spinal cord, with upregulation of genes linked the NF-kB, TREM2, APOE, and phagocytic pathways. In the motor cortex, only 42 genes were differentially expressed favouring down regulation of proinflammatory genes.

In the spinal cord of MND cases, 46 genes were associated with survival time (similar numbers correlated with longer and shorter survival). In motor cortex, 47 genes were associated with patient survival, 30 of which were associated with longer survival.

The results from frozen spinal cord correlated well with previous results from FFPE tissue and archival RNA seq data from other laboratories.

Conclusion:
The spinal cord appears to be a more inflammatory environment, compared to motor cortex. Immunity-associated gene expression pathways differ between control and MND tissues and correlate with the rate of disease progression. Further work will validate these changes using immunohistochemistry.

HCB-13: Investigation of the Caprin-1 Interactome to Gain Insight into the Proteome of Stress Granules

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Background:
Cytoplasmic stress granules are dynamic non-membranous foci containing translationally arrested mRNA and RNA binding proteins which form in response to a variety of cellular stresses. A leading hypothesis in the field is that stress granules evolve into the characteristic pathological inclusions observed in ALS patient neurons. Recent studies have begun to elucidate the proteome of stress granules by interrogating the interactome of G3BP1 using immunoprecipitation coupled with mass spectrometry. While this has provided tremendous insight, multiple bait proteins are required to fully capture the complete stress granule proteome. Additionally, prior studies were performed using either overexpression or the integration of tagged sequences, each of which may perturb protein stoichiometry or structure. Characterization of stress granules at physiological levels and native protein structure is lacking. Moreover, G3BP1 is now known to be regulated by TDP-43, and thus these prior efforts may not be able to adequately inform on the hypothesis that stress granules evolve to pathological inclusions in ALS. Recent studies within our group demonstrated the presence of Caprin-1 containing cytoplasmic inclusions. Additionally, others determined that Caprin-1 co-localized with pTDP-43 in ALS spinal cord motor neurons.

Objectives:
To gain insight into the proteome of Caprin-1 containing inclusions, we employed immunoprecipitation of endogenous Caprin-1, which is not impacted by TDP-43 localization or mutation, to further define the stress granule proteome.
Methods:
HeLa cells were either unstressed or subjected to sodium arsenite stress and processed for immunoprecipitation with either Caprin-1 or IgG-coated Dynabeads. Peptides were detected using unbiased mass spectrometry and proteomics analyses were used to interrogate the Caprin-1interactome.
Results:
Overall, we identified 1500 proteins that interacted with Caprin-1 in basal and stressed conditions. Among these, 281 and 326 proteins were considered high-confidence Caprin-1 interactors under stressed and unstressed conditions, respectively. Based on gene ontology analyses, interactors under stressed conditions were annotated to the ribosome, spliceosome, and RNA transport. We chose 12 proteins to further study that have not been previously localized to stress granules. Of these, 4 validated as novel interactors of Caprin-1 and localized to arsenite-induced stress granules: Ankyrin repeat and KH domain-containing protein 1 (ANKHD1); Talin-1 (TLN1); Gem-associated protein 5 (GEMIN5); and U5 small nuclear ribonucleoprotein 200 kDa helicase (SNRNP200). We also validated these stress-induced interactions in SH-SY5Y neuronal-like cells and also determined that SNRNP200 associated with osmotic and thermal stress-induced granules. These data indicate that SNRNP200 is a novel stress granule component. Current efforts are investigating SNRNP200 subcellular localization in ALS spinal cord and motor cortex.
Conclusions:
Our findings provide the first Caprin-1 protein-based interactome, novel cytoplasmic stress granule components, and potential pathogenic pathways to further investigate in ALS.
Support:
Funding provided by the ALS Canada/Brain Canada Arthur J. Hudson Translational Team Grant (CVV, RB).

HCB-14: Nuclear localization signal deletion differentiates between TDP-43 cytoplasmic and nuclear phase separation

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Background:
The main pathophysiological feature in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are the protein aggregates that form in the cytoplasm of neurons and glia. These aggregates contain TDP-43 proteins in 97% of ALS cases, most of which are sporadic [1], and in approximately half of FTD cases [2]. TDP-43 is a DNA and RNA binding protein. In cells, it circulates between the nucleus and the cytoplasm and participates in mRNA transfer to the cytoplasm [3]. One of the questions that raise dilemmas is whether TDP-43 aggregation causes loss of function (LOF), gain of function (GOF), or both [4,5].

Objectives:
In this study, we investigated protein interactions in the proximal environment of wild type TDP-43 (TDP-43wt) and TDP-43 lacking the nuclear localization signal (dNLS-TDP-43).

Methods:
The labelling of TDP-43wt and dNLS-TDP-43 interacting partners was conducted by the BioID2 proximity labeling method. Three HEK Flp-In cell lines were established, namely the control cell line (BioID2), the cell line with inducible wild-type TDP-43 construct cloned in vicinity of the coding sequence for biotin ligase (BirA) (BioID2-TDP-43wt) and a cell line with TDP-43dNLS and BirA (BioID2-dNLS-TDP-43). Following the induction of BirA activity the biotynilated proteins were pulled down by streptavidin magnetic beads. The pull-down proteins were detected by silver staining, Western blot, and mass spectrometry (MS). The MS results gave a list of unique TDP-43wt and TDP-43dNLS interactors, these were validated by immunocytochemistry, Western blot, and proximity ligation assay (PLA) and their cellular localization and function assessed by DAVID bioinformatics analysis.

Results:
The proximity-dependent biotin identification method (BioID2), followed by MS, revealed that wild type TDP-43 primarily interacts with proteins of ribonucleoprotein, spliceosomal complexes, and subnuclear structures - paraspeckles. Consistent with their nuclear localization, functions of the identified TDP-43wt interaction partners are associated with transcriptional regulation, splicing, and gene expression. In contrast to wild type TDP-43, dNLS-TDP-43 interactors were components of cytoplasmic stress granules (SG) and P-bodies. Their role is therefore associated with translational repression or degradation of the mRNA molecules.

Discussion:
Our results show that the pathological mechanisms which lead to ALS may involve the loss of regulatory functions associated with transcription and/or paraspeckle function. On the other hand, the increased association of mutant dNLS-TDP-43 with proteins of SGs and P-bodies indicates that they may also have an important role in the mechanism of neurodegeneration in ALS.

Acknowledgments:
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[1] Arai et al. Biochem. Biophys. Res. Commun. 351 (2006)
[2] Neumann et al. V.M.-Y. Lee, Science 314 (2006)
[3] Prpar Mihevc et al. Brain J. Neurol. (2016)
[4] Dormann et al.Trends Neurosci. 34 (2011)
[5] Prpar Mihevc et al. Sci. Rep. 6 (2016)

HCB-15: Nuclear pore complex quality control in C9orf72 and sporadic ALS

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A G4C2 hexanucleotide repeat expansion (HRE) in the C9orf72 gene is causative of ALS and FTD. Recently, we have demonstrated that pathologic repeat RNA species arising from this HRE leads to a reduction in the nuclear levels of 8 specific components (nucleoporins, Nups) of the nuclear pore complex (NPC) in C9orf72 induced pluripotent stem cell (iPSC) derived spinal neurons (iPSNs) and postmortem patient tissue. Recent data suggests that this specific Nup pathology is also observed in a subset of sALS iPSNs. Here, we have now uncovered the underlying mechanisms that lead to this Nup loss and disruption of NPCs in neurodegeneration. We provide evidence that CHMP7 and VPS4, components of an ESCRT pathway linked to NPC quality control, are dramatically increased in C9orf72 and some sALS iPSN nuclei prior to the emergence of Nup alterations. Confirmatory evidence of this pathology is also present in postmortem human brain tissue. Consistent with a role in regulating Nup levels within NPCs, knockdown of CHMP7 mitigates disease associated injury and restores the nuclear levels of specific Nups in human neurons. Additionally, by specifically inhibiting the nuclear export of CHMP7, we can trigger Nup reduction. Thus, our data support a role for disruptions in CHMP7 and ESCRT-III mediated Nup homeostasis in mammalian nuclei and C9orf72 and sALS disease pathogenesis. Overall, this study provides evidence of a role for this novel NPC surveillance pathway in the initiation of neurodegeneration.

HCB-16: Proteins binding RNA transcripts from C9orf72 gene mutation

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Background:
Expanded repeat mutations are underlying pathology of multiple neurodegenerative diseases including ALS/FTD. Expanded repeat RNA transcripts are proposed to have toxic effect on cells by sequestering proteins important for cell function. Sense RNA transcripts from C9orf72 mutation have been studied extensively (1), while there is less data on antisense RNA transcripts, which are also present in disease pathology (2).

Objectives:
Identification of proteins interacting with antisense RNA transcripts from the C9orf72 gene mutation and defining their role in pathology of ALS and FTD.

Methods:
RNA pull-down assay was performed using long RNA constructs (32xC4G2). Proteins were identified by mass spectrometry. RNA-protein colocalization in cell was studied using RNA fluorescence in situ hybridization and immunofluorescent staining (IF) of proteins. RNA-protein proximity ligation assay (PLA) was optimized and used in analysis of RNA-protein interaction. Control and C9orf72 mutation positive fibroblasts, lymphoblasts and iPSCs were used. Aminoacylation assay was performed on cells for determination of tRNA charging levels.

Results:
Proteins identified as interactors of antisense RNA from C9orf72 mutation are involved in various cell processes such as RNA processing, protein synthesis and cytoskeleton stability. Among identified proteins was phenylalanine tRNA synthetase (FARS). We found colocalization of FARS with antisense RNA in cells by RNA-FISH/IF as well as increase in signal in C9orf72 mutation positive cell lines compared to control by RNA-protein PLA. By preforming aminoacylation assay we observed increase in uncharged tRNA levels in C9orf72 mutation positive lymphoblasts relative to control.

Discussion:
This study reveals novel proteins interacting with antisense RNA repeats and for the first time shows their colocalization in cells by RNA-protein PLA. We have focused on FARS, as aminoacyl tRNA synthetases are being increasingly mentioned in disruption of the nervous system (3). We have shown changes in tRNA aminoacylation in C9orf72 mutation positive lymphoblasts indicating possible disruption in function of FARS protein connected to C9orf72 mutation.

References:
1. S. Vatovec, A. Kovanda, B. Rogelj, Neurobiol. Aging 35 (2014) 2421.e1-2421.e12.
2. A.D. Gitler, H. Tsuiji, Brain Res. 1647 (2016) 19–29.
3. J. Ognjenović, M. Simonović, RNA Biol. 15 (2017) 623–634.

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HCB-17: Proteome-Wide Degradation Dynamics in Patient Motor Neurons Reveal a Novel Functional Interaction Between VCP and SOD1

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Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the selective death of motor neurons (MNs). Mutations in SOD1 that account for up to 20% of all genetic ALS cases lead to neurotoxicity through gain-of-function effects involving the accumulation of misfolded SOD1 protein. While defects in mitochondrial metabolism, trafficking, cytoskeletal homeostasis and proteostasis have been implicated in mutant SOD1-dependent MN degeneration, the precise mechanism by which misfolded SOD1 protein impairs these pathways remains unclear. Here, we used an induced pluripotent stem cell (iPSC) isogenic model system coupled to quantitative mass spectrometry (MS) to investigate this interaction. We first defined the kinetics and state of mutant SOD1 protein. We find that patient MNs carrying the SOD1 A4V mutation exhibit consistently higher levels of disordered, soluble SOD1 protein relative to isogenic control MNs. At the same time, disordered SOD1 progressively accumulates in the insoluble fraction after 25 days in vitro, suggesting that protein degradation mechanisms become progressively impaired. Using stable isotope labeling with amino acids in cell culture (SILAC)-based MS we monitored the proteome wide degradation dynamics over 3-weeks and found that patient MNs consistently accumulate more “older” proteins. These proteins that are degraded at a slower rate, include other known ALS causal genes and are enriched for protein folding, trafficking and microtubule homeostasis pathways. Amongst the most continently older proteins we found was VCP, which plays a key role in proteasome-dependent degradation and autophagy. VCP itself can cause familial ALS by predominantly loss of function mutations. We find that the interactome of older VCP protein is significantly altered in SOD1 A4V MNs in vitro, while VCP forms aggregates in SOD1 A4V ALS postmortem patient tissue in vivo. Lastly, reduction of VCP expression exacerbates, while overexpression of VCP rescues mutant SOD1 toxicity respectively, in a C. elegans model in vivo. Our results suggest that VCP dysfunction mediates SOD1-dependent degeneration and highlights impaired protein degradation as an underlying mechanism linking distinct genetic subtypes of ALS.

HCB-18: Unique inflammatory transcriptional profiles distinguish long from short disease duration ALS.

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Aim:
Amyotrophic Lateral Sclerosis (ALS) is a progressively fatal and highly heterogeneous neurodegenerative disease. Significant variation exists across the genetic background and phenotypic presentation, particularly with respect to survival (1). The identification of TDP-43 protein aggregates as a pathological hallmark of ALS has established the condition as a proteinopathy (2). As TDP-43 is ubiquitously expressed and regulates transcription and splicing, understanding the transcriptional profiles associated with distinct ALS clinical phenotypes, particularly those associated with neuro-resilience, is a critical yet understudied area.

Methods:
We carried out transcriptional profiling on motor cortex samples from a cohort of 20 sporadic ALS (sALS) cases stratified by disease duration. The genetic status of all samples was assessed by whole genome sequencing or C9orf72 repeat-prime polymerase chain reaction (PCR) and the application of an extended 49-gene panel. ALS cases were compared with 11 age and gender matched non-neurological control samples. Transcriptional profiling was carried out using NanoString (3), a novel molecular barcoding technology comprised of 760 genes associated with neurodegeneration. Bioinformatic analysis of differential gene expression was conducted using DESeq2.

Results:
Comparison of the long versus short sALS disease duration groups identified upregulation of pro-inflammatory and innate immune response gene sets as well as downregulation of organelle, vesicle localization and synapse gene sets. Two outlier samples of long disease duration were identified which were also distinguished from the sALS cohort by autoimmune comorbidity. Cell type specific analyses of the differentially expressed genes were performed using the Barres Brain RNA-seq data (4). Upregulated genes were associated with microglial and endothelial cells and downregulated genes were associated with synapses.

Conclusions:
Our data demonstrate that inflammatory pathway dysregulation distinguishes long and short disease duration sALS cases. We are now validating our findings using RNA in-situ hybridisation probes for selected microglial and glymphatic system genes. Understanding the molecular basis of factors which confer neuro-resilience will facilitate the development of biomarkers, targeted disease therapies and enable the stratification of future trials to test these candidates.

References:
1. Westeneng H, Debray T, Visser A, Van Eijk R, Rooney J, Calvo A et al. Prognosis for patients with amyotrophic lateral sclerosis: Development and validation of a personalised prediction model. The Lancet Neurology, (2018) 17(5), 423-433.
2. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science (New York, N.Y.). (2006) 314(5796): 130–3. Retrieved from www.jstor.org/stable/20031485
3. https://www.nanostring.com/products/gene-expression-panels/ncounter-neuropathology-panels
4. Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD et al. Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse (2016). Neuron (Cambridge, Mass.), 89(1), 37-53. http://www.brainrnaseq.org

HCB-19: Biphasic nucleolar stress in C9orf72 and sporadic ALS spinal motor neurons correlates with TDP-43 mislocalization

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Nucleolar stress has been implicated in pathology and disease pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) both from repeat expansion of GGGGCC in C9orf72 (C9-ALS/FTD) and sporadic ALS. Previously we reported that antisense RNA transcripts are unique in C9-ALS because of their nucleolar localization in spinal motor neurons and correlation with TDP-43 mislocalization, the hallmark of proteinopathy of ALS and FTD. Here we further studied postmortem spinal cord tissue in 11 control, 11 C9-ALS and 11 SALS nervous systems to determine if there is other evidence of nucleolar stress. We found nucleolar shrinkage in both C9-ALS and SALS spinal motor neurons. We found this in neurons both with and without TDP-43 mislocalization or nucleolar antisense RNA foci. Surprisingly, nucleolar shrinkage was greatest in neurons without these hallmarks, findings consonant with other reports. Thus, nucleolar stress in both C9-ALS and SALS spinal motor neurons appears to be biphasic -- shrinkage is greatest before the other main pathological hallmarks appear.

HCB-20: C9orf72 frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS)—Using patient cells and CRISPR to reveal therapeutic approaches

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Expansion of the GGGGCC hexanucleotide repeat in the C9orf72 gene is the most frequent known genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS)1–3 (termed C9-FTD/ALS). Although the cellular dysfunction caused by this disease is multifactorial4, targeting the gene itself by CRISPR/Cas9 editing could be curative5. The most pressing challenge to implementing this technology effectively is identifying edits that neutralize the C9orf72 mutation without introducing unintended cellular dysfunction. We have taken three approaches to editing the C9orf72 gene in human iPSC cells: (1) bi-allelic excision of non-coding DNA harboring the repeat expansion region, (2) allele-specific excision of the mutant allele containing the repeat expansion, (3) regulatory region disruption to selectively silence the C9orf72 repeat expansion. We have additionally engineered our cell lines with inducible neuron-specific transcription factors as a high-throughput method for producing disease-relevant cell types (cortical and motor neurons). By studying the effects of these genetic manipulations in induced neurons we not only interrogate gene editing approaches, but also advance our understanding of the normal regulation of the C9orf72 gene.

HCB-21: HDAC inhibitors counteract the effects of dipeptide repeats proteins on nucleocytoplasmic transport

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Background:
A repeat expansion mutation in the C9ORF72 gene is the most commonly known genetic cause of both sporadic and familial cases of ALS and frontotemporal dementia (FTD), a related neurodegenerative disease. The expansion mutation produces five dipeptide-repeat proteins (DPRs), including poly proline-arginine (PR), glycine-arginine (GR) and glycine-alanine (GA). While the mechanistic determinants of DPR-mediated neurotoxicity remain incomplete, substantial evidence suggests that the disruption of nucleocytoplasmic transport contributes to pathology [1,2,3]. Therefore, characterizing these nucleocytoplasmic transport disturbances and determining the relative contribution of each DPR is needed in order to identify strategies to restore nucleocytoplasmic transport in DPR expressing cells.

Objectives:
To evaluate the effects of DPRs on all major nucleocytoplasmic transport pathways, and identify compounds that counteract these effects.

Methods:
We generated a series of nucleocytoplasmic transport biosensors composed of the green fluorescent protein fused to different classes of nuclear localization signals (NLSs) and nuclear export signals (NESs). Using these "biosensors" in conjunction with high content automated microscopy, we investigated the role of the three most neurotoxic DPRs (PR, GR, and GA) on 7 nuclear import and 2 export pathways. Furthermore, we carried out a small molecule screen to identify compounds that counteract the DPR-mediated translocation of the biosensors.

Results:
In addition to other DPRs, we found that PR had pronounced inhibitory effects on all nucleocytoplasmic transport pathways. Several molecules, primarily HDAC inhibitors, were identified that restored classical nucleocytoplasmic transport in PR-expressing cells and increased their cellular viability.

Discussion:
We found that DPRs disrupt multiple nucleocytoplasmic transport pathways and identified small molecules that counteract these effects and increase cell survival, suggesting restoration of nucleocytoplasmic transport in PR-expressing cells may confer therapeutic effects. Many of these small molecules are HDAC inhibitors, supporting previous studies that show HDAC inhibition inducing therapeutic affects in neurodegenerative models [4].

References:
1. Jovicic A, Mertens J, Boeynaems S, et al. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. Nat Neurosci. 2015;18(9):1226-1229.
2. Boeynaems S, Bogaert E, Michiels E, et al. Drosophila screen connects nuclear transport genes to DPR pathology in c9ALS/FTD. Sci Rep. 2016;6:20877.
3. Freibaum BD, Lu Y, Lopez-Gonzalez R, et al. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. Nature. 2015;525(7567):129-133.
4. Saha RN, Pahan K. HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. Cell Death Differ. 2006;13(4):539-550.

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HCB-22: HNRNPH and its localisation to nuclear G4C2 foci and cytoplasmic stress granules of C9orf72 amyotrophic lateral sclerosis

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Background:
Hexanucleotide (G_4C_2) repeat expansion, spanning from several hundred to several thousand repeats, in the first intron of C9orf72, is the most common known cause of amyotrophic lateral sclerosis (ALS) and an etiological point of origin of several divergent pathological pathways [1,2]. One of the interaction partners of G_4C_2 expansion is heterogeneous nuclear ribonucleoprotein H (HNRNPH) [3]. We have detected its colocalisation with the pathological G4C2 foci in ALS and frontotemporal dementia (FTD) patient brains [4] and demonstrated that these sense foci form paraspeckle-like bodies binding also HNRNPH [5].

Objectives:
Define the role of individual HNRNPH domains on its localization into G_4C_2 nuclear foci and cytoplasmic stress granules (SG).

Methods:
Series of protein fragments was constructed based on HNRNPH1 domain structure including mutated quasi RNA-recognition motif (qRRM) domains. Colocalization of fragments and G_4C_2 foci in co-transfected cells (individual fragments and 72×G_4C_2) was detected by fluorescence in situ hybridization (FISH) coupled with immunocytochemistry. SG formation was inducted by arsenite in cells after the transfection with HNRNPH1 fragments. Their colocalization was detected by immunofluorescence staining (PABP marking SG). The RNA-binding domain map (RBDmap) of HNRNPH1 was constructed and a model of the HNRNPH1 binding to RNA generated with Coot and visualized with Pymol.

Results:
All three individual qRRM's are sufficient for sequestration of HNRNPH1 into G_4C_2 foci. Endogenous HNRNPH exhibits nucleocytoplasmic shuttling and abundantly localizes to SGs upon induction of oxidative stress. Two of three qRRM domains are required for colocalization with SG markers. The RBDmap showed insight into native HNRNPH1-RNA interactions in living cells and the binding model the possible mode of HNRNPH1 binding to specific RNAs.

Discussion:
Due to large abundance of various mRNAs contained in G_4C_2 foci and mature SGs the results are interesting and imply different specificities or binding activities of otherwise related domains. The nuclear foci share a group of interacting proteins with SGs and their simultaneous presence in neurons might have further pathological effects in ALS and FTD.

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[1] Renton et al. Neuron 72 (2011) 257–268.
[2] DeJesus-Hernandez et al. Neuron 72 (2011) 245–256.
[3] Haeusler et al. Nat. Rev. Neurosci. 17 (2016) 383–395.
[4] Lee et al. Cell Rep. 5 (2013) 1178–1186.
[5] Bajc Česnik, Darovic, Prpar Mihevc et al. J. Cell Sci. 132 (2019).

HCB-23: Identifying pathomechanisms in iPSC-derived cortical neurons from C9orf72-associated ALS/FTD and FTD patients

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Background:
A hexanucleotide repeat expansion in the C9orf72 gene is the most frequent cause of both Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). While the C9orf72 mutations causes a degeneration of motor neurons in the spinal cord and motor cortex in ALS (C9-ALS), it causes a degeneration of cortical neurons of the frontal and/or temporal lobe in FTD (C9-ALS). About 10-15% of patients from both groups suffer from both C9-ALS and C9-FTD (C9-ALS/FTD).
Apart from a haploinsufficiency of the C9orf72 protein, the C9orf72 hexanucleotide repeat expansion leads to the expression of dipeptide repeat proteins (DPRs), of which poly-GR, poly-PR, and poly-GA are believed to be toxic. Several pathomechanisms caused by the C9orf72 mutation have been identified, such as impaired autophagy, excitotoxicity, ER stress, apoptosis, stress granule formation, and mitochondrial dysfunction, which have all been shown in C9-ALS and C9-ALS/FTD induced pluripotent stem cell (iPSC)-derived motor neurons. However, few studies have tested these mechanisms in iPSC-derived cortical neurons and in neurons from C9-FTD patients.

Aim:
The aim of this study is to phenotype iPSC-derived cortical neurons from C9-ALS/FTD, C9-ALS, and C9-FTD patients to identify pathomechanisms caused by the C9orf72 hexanucleotide repeat expansion.

Methods:
In this study, we differentiated cortical neurons derived from iPSCs from C9-ALS/FTD, C9-ALS, and C9-FTD patients. We phenotyped day 55 neurons using western blot, immunocytochemistry, functional assays, and calcium imaging, to study autophagy impairment, ER stress, stress granules, mitochondrial dysfunction, cell death, and altered excitability.

Results:
We identified a significantly increased number of stress granules in C9-ALS/FTD, but not in C9-FTD cortical neurons treated with sodium arsenite. This altered stress response might be caused by a poly-PR and poly-GR-associated impairment of translation. Furthermore, we found a disruption in mitochondrial function in C9-FTD cortical neurons, which might be caused by poly-GR associated oxidative stress. We also identified small changes in ER stress and AMPA receptor expression in C9-FTD, as well as a subtle increase in apoptosis in all patient lines.

Conclusion:
This study identifies C9orf72 mutation-induced pathomechanisms in cortical neurons derived from ALS, ALS/FTD, and FTD patients, such as increased stress granule formation in C9-ALS/FTD cortical neurons and impaired mitochondrial function in C9-FTD cortical neurons. This provides understanding of disrupted mechanisms in an early disease stage of frontotemporal dementia. It also provides information on potential differences in pathomechanisms in motor neurons and cortical neurons, in ALS and FTD.

HCB-24: Impaired DNA damage response as the upstream event in FUS-ALS pathophysiology does not lead to a higher cancer prevalence in patients

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Background:
Mutations in Fused in Sarcoma (FUS) were shown to be causative in up to 5% of genetic ALS cases and lead to both, early and late disease onset. Proper shuttling of the FUS protein between nucleus and cytoplasm is essential for its physiological function in DNA damage response (DDR) signaling, splicing regulation and in stress granule formation. Heterozygous FUS-NLS (nuclear localization sequence) mutations are believed to impair this nucleocytoplasmic transport resulting in cytoplasmic FUS aggregates, which are pathological hallmarks of FUS-ALS. However, it remains to be studied if different FUS mutations correlate with specific phenotypic changes. The clear upstream event in the pathophysiology of FUS-ALS leading to MND remains enigmatic.
Objectives:
We aimed to decipher the biomolecular changes that drive the disease onset and progression of FUS-ALS via an up to date in vitro human model system. Based on these results, a phenotypic assessment of newly compiled and previously published FUS-ALS patients was initiated.
Methods:
We established a human induced pluripotent stem cell (hiPSC) model derived from fibroblasts from affected patients with evident FUS mutation. CRISPR-Cas9 was applied to create isogenic controls and tag the endogenous FUS protein. A number of assays were used to study DDR signaling and organelle movement, which included laser-ablation and TIRF live-cell imaging microscopy. Via a multicenter cohort-study we cross-sectionally reviewed the patient history of FUS-ALS patients and identified 36 new patients. A meta-analysis of clinical characteristics was conducted by adding data of previously published cases.
Results:
Using hiPSCs-derived spinal motor neurons (MNs), we show that impairment of poly(ADP-ribose) polymerase (PARP)-dependent DDR signaling due to mutations in the FUS-NLS induces additional cytoplasmic FUS mislocalization. Furthermore, we observed a secondary distal axonal phenotype, which was dependent on a sufficient PARP DDR function and led to axonal deterioration in the FUS mutants. Although there was evidence for genomic instability in both, in vitro MNs and FUS-ALS patient post mortem tissue, no evidence of an increased cancer burden was found in the multicenter analysis. However, a genotype-phenotype correlation of clinical-neurological features could be accomplished, which indicated the P525L change to lead to the most severe phenotype and atypical ALS while the R521 locus is most frequently affected, but results in milder – most often spinal – disease courses.
Discussion:
Collectively, our work suggests that a key pathophysiologic event in FUS-ALS is upstream of aggregate formation within the DDR pathway. We propose a vicious cycle resulting in progressive cytoplasmic FUS accumulation and loss of its nuclear function leading to neurodegeneration and FUS aggregate formation. Targeting DDR signaling could be a promising disease modifying option in (FUS-) ALS. We report the largest genotype–phenotype correlation of FUS-ALS, which enables a careful prediction of the clinical course in newly diagnosed patients.

HCB-25: Investigating neuroinflammatory dysregulation and its implications for disease phenotype and progression in C9orf72 post-mortem tissue

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Background:
Recent studies have shown that protein products of the ALS/FTD-associated genes C9orf72, TBK1 and PGRN are involved in inflammatory pathways (1). Indeed, dysregulation of these pathways is noted to correlate well with clinical phenotype (2-3). Unfortunately, despite these links, clinical trials exploring the use of anti-inflammatory drugs for ALS therapy have been unsuccessful (4). This may be attributed, at least in part, to non-specific mechanisms of drug action, lack of appropriate patient stratification and/or trial endpoints, as well as the bias of preclinical studies toward SOD1 mouse models, while SOD1 mutations account for only 2% of ALS cases and are not at all associated with FTD (5-6). Thus, further characterisation of neuroinflammation along the ALS-FTD spectrum is warranted to understand how these pathways can be more specifically targeted to harness their therapeutic potential.
Objective:
To characterise neuroinflammatory profiles along the ALS-FTD spectrum and explore correlations with symptoms and pathological features.
Methods:
We used techniques such as immunohistochemistry and immunofluorescence to characterise neuroinflammatory profiles in a cohort of deeply clinically phenotyped human post-mortem tissue.
Results:
Here we show how neuroinflammatory pathway dynamics vary across a clinically heterogeneous cohort, all with the same genetic mutation (C9orf72 hexanucleotide repeat expansion), and explore how these dynamics correlate with motor and cognitive symptoms, as well as pathological features.
Discussion:
Our data, taken together, serve as a basis for future research to better our understanding of neuroinflammatory dynamics along the ALS-FTD spectrum and highlight the potential to target dysregulated pathways based on individual inflammatory profiles (7).
References:
(1) Gao F-B, Almeida S, Lopez-Gonzalez R. Dysregulated molecular pathways in amyotrophic lateral sclerosis-frontotemporal dementia spectrum disorder. EMBO J. 2017;36(20):2931-2950.
(2) Oeckl P, Weydt P, Steinacker P, et al. Different neuroinflammatory profile in amyotrophic lateral sclerosis and frontotemporal dementia is linked to the clinical phase. Journal of Neurology, Neurosurgery & Psychiatry. 2019;90(1):4-10.
(3) Brettschneider J., Toledo J. B., Van Deerlin V. M., Elman L., McCluskey L., Lee V. M., et al. . (2012). Microglial activation correlates with disease progression and upper motor neuron clinical symptoms in amyotrophic lateral sclerosis. PLoS ONE 7:e39216. 10.1371/journal.pone.0039216.
(4) Petrov D, Mansfield C, Moussy A, Hermine O. ALS Clinical Trials Review: 20 Years of Failure. Are We Any Closer to Registering a New Treatment? Front Aging Neurosci. 2017;9:68-68.
(5) Taylor JP, Brown RH, Cleveland DW. Decoding ALS: from genes to mechanism. Nature. 2016;539(7628):197-206.
(6) Tang B. Amyotrophic lateral sclerosis disease modifying therapeutics: a cell biological perspective. Neural Regeneration Research. 2017;12(3):407-408.
(7) Fyfe I. Do inflammatory profiles explain ALS-FTD spectrum? Nat Rev Neurol. 2018;14(11):634.

Acknowledgments
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HCB-26: Investigating the molecular mechanisms of mutant C9orf72 human iPSC-derived astrocyte-mediated motor neuron deficits.

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Background:
Amyotrophic Lateral Sclerosis (ALS) is a progressive, incurable and invariably fatal neurodegenerative condition characterised by loss of motor neurons. Over the last two decades, there are multiple lines of evidence from pathology, genetics and experimental systems that implicate a central role for non-neuronal cells, astrocytes in particular, with ALS pathogenesis. This is not surprising given the intimate structural and functional association of astrocytes with, inter alia, the synapse and vasculature. Yet, the nature of this astrocyte-neuronal crosstalk remains understudied. Harnessing the power of genetic discoveries combined with human iPS cellular platforms and gene editing technologies, we seek to better understand the non-cell autonomous effect of astrocytes carrying the C9orf72 mutation – the commonest genetic cause of ALS – on motor neurons.

Methods:
We generate human astrocytes and motor neurons from three pairs of independent patient-derived mutant C9orf72 (mC9) iPSC lines and their corresponding CRISPR-Cas9 gene-edited isogenic controls. Having isogenic controls allows causality to be assigned to any given phenotype and overcomes transcriptional heterogeneity across iPSC lines. We utilise these cells to investigate astrocyte-mediated neuronal toxicity and changes in secretome profiles.

Findings:
By implementing in-house published protocols, highly enriched functional astrocytes from control, mC9 and gene-edited iPSCs are generated, as evidenced by expression of astrocyte markers (S100β+/GFAP+), uptake of extracellular glutamate and calcium wave propagation under stimulation. Notably, mutant astrocytes recapitulate key aspects of C9orf72-related ALS pathology with nuclear RNA foci and poly-GP dipeptide repeats that are abolished in isogenic controls. In co-culture experiments with wild-type motor neurons, mC9 astrocytes induce physiological defects characterised by temporal loss of neuronal excitability (Zhao C, et al. 2019) and impart neuronal morphological deficits. Additionally, our preliminary data demonstrates that C9-astrocytes are toxic to motor neurons via secreted factors by inducing neurite outgrowth deficits, an effect that is reversed in isogenic controls.

Interpretation:
The toxic effect of the C9-astrocyte secretome could be the result of release of astrocyte-derived neurotoxins or impaired secretion of trophic factors and hence reduced capability to support neurons. By using unbiased mass spectrometric analysis and molecular techniques, we aim to identify the underpinning molecular mechanisms of astrocyte-mediated neuronal toxicity. This will potentially lead to the identification of putative neuroprotective targets as well as novel biomarkers.

Reference:
Chen Z, Devlin AC, Chouhan AK, Selvaraj B, Stavrou M, ...& Chandran S. Mutant C9orf72 human iPSC-derived astrocytes cause non-cell autonomous motor neuron pathophysiology. Glia 2019 doi: 10.1002/glia.23761

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Regenerative Neurology Clinic, University of Edinburgh, UK.

HCB-27: Investigating the Pathological Contribution of the Skeletal Muscle to Neuromuscular Junction Dysfunction in ALS, Using a Patient iPSC-derived Phenotypic Model

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Background:
While there have been advances in Amyotrophic Lateral Sclerosis (ALS) research in the past decade, the cause of disease onset remains elusive. Neuromuscular junction (NMJ) dysfunction has been identified as an early event in ALS pathology. However, which tissue type induces NMJ disruption, be it the motoneuron or skeletal muscle, is not understood. The specific susceptibility of motoneurons in ALS led early researchers to adopt a "neurocentric" approach in investigating the disease. However, recent findings report the pathological involvement of the skeletal muscle in ALS onset and progression (1).

Objectives:
This study sought to characterize ALS skeletal muscle and subsequently, its possible pathological contribution to NMJ dysfunction.

Methods:
ALS skeletal muscle was obtained by differentiating induced pluripotent stem cells (iPSCs) derived from patients. The ALS skeletal muscle (ALS-SKM) was then characterized morphologically and functionally in comparison to healthy control (WT).

Results:
Per imaging and fusion index quantification, ALS skeletal muscle progenitors had comparatively delayed and reduced fusibility. Immunocytochemistry staining also showed little to no acetylcholine receptor clustering in ALS muscle cultures. Additionally, functional assessment of the diseased muscle indicated a significantly lower contractile force coupled with reduced contraction fidelity upon stimulation in comparison to healthy muscle. Mitochondrial analysis also revealed a reduced inner membrane potential as well as altered cell metabolism in ALS skeletal muscle. The iPSC-derived muscle, from patient or WT, are currently being mixed-matched in co-culture with iPSC-derived motoneurons (MN), from patients or WT. The role of ALS-SKM in generating NMJ functional pathology is being evaluated in comparison with the role of ALS-MN. Preliminary results indicate a significant contribution of ALS-SKM in NMJ dysfunction even in combination with WT-MN, suggesting muscle pathology is an active player in the initiation of NMJ deterioration in ALS etiology. Collectively, these results agree with both clinical and transgenic model studies that reported altered NMJ integrity in ALS.

Discussion:
This study demonstrates that the ALS skeletal muscle possesses some pathological alterations that may detrimentally affect NMJ integrity. Moving forward, the ALS-iPSC NMJ models will be utilized in characterizing the nature of the ALS synapse, in the quest of unraveling events that may induce NMJ instability. Finally, this model holds the promise of serving as a viable platform for drug studies, in an effort to hasten the ALS drug discovery process.

References:
(1) Campanari et al (2019). Diagnostic challenge and neuromuscular junction contribution to ALS pathogenesis. Frontiers in Neurology, 10, 68.

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HCB-28: Investigating the properties of control iPSCs lines to differentiate into spinal motor neurons and to form neuromuscular junctions in 2D

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that selectively affects cortical, brainstem and spinal motor neurons (MNs). While most of the reported cases of ALS are sporadic (sALS), 5 to10% are familial (fALS), caused by mutations in known ALS-linked genes. Disruption of the neuromuscular junction (NMJ) is an early event in the development of the disease, however, the precise mechanisms leading to NMJ dysfunction and subsequent denervation remain unknown. Understanding the underlying molecular and cellular mechanisms of such a crucial event in the onset of the disease is critical in an effort to develop efficient treatments for the disease. With this in mind, we developed a 2D NMJ model using human primary myoblasts differentiated into skeletal muscle and induced pluripotent stem cell (iPSCs)-derived MNs from 2 different healthy individuals. Characterization of the differentiated cells was performed by immunofluorescence (IF) with specific markers for both motor neurons (e.g. CHAT, HB9, Islet and SMI-32) and skeletal muscle (e.g. MyHC, Desmin, SAA). This analysis was complemented by quantifying levels of the corresponding genes by qPCR. With MNs established, we investigated whether these neurons were capable of forming NMJs by co-culturing human skeletal muscle with iPSC-derived MNs. The successful formation of NMJs in co-cultures was confirmed by α-bungarotoxin staining and colocalization between SV2 and SMI-32. This model will be used in the future to study NMJ stability in ALS and to build a high-throughput screening assay for small molecules by using human iPSC-derived motor neurons from patients with both fALS and sALS.

HCB-29: MicroRNA dysregulation as a driver of WNT activation and motor neuron degeneration in ALS

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Background:
Underlying mechanisms driving motor neuron (MN) degeneration in ALS remain poorly understood due to complex disease heterogeneity. Consequently, development of effective treatments remains elusive. Recent studies identify unifying disruption to key MN homeostatic functions such as RNA processing, in both sporadic and familial ALS. It is yet unclear how such defects drive MN degeneration in ALS.

Objectives:
In this study, we take a top-down approach to identify dysregulation of cell signalling pathways, and investigate the mechanisms driving ALS-associated MN degeneration.

Methods:
Patient-derived induced pluripotent stem cells (iPSCs) harbouring mutations in the FUS gene were differentiated into motor neurons alongside isogenic and independent healthy control cells. We performed detailed phenotypic and transcriptomic analyses of iPSC-derived MNs, alongside computational analyses of existing ALS datasets to discover key signalling pathways that drive neurodegeneration.

Results:
Our transcriptomic analysis identified several dysregulated pathways in FUS-ALS MNs, including activation of cell cycle, STAT3, p38 MAPK, WNT and p53. Pharmacological inhibition of WNT yielded the highest improvement in FUS-ALS MN survival. Accordingly, we confirmed activation of WNT in FUS ALS MNs using an array of biochemical assays including luciferase assays, western blots and quantitative RT-qPCR of WNT target genes and ligands. Further, we discovered that dysregulation of a MN-enriched microRNA is responsible for WNT activation, and demonstrate that modulation of this microRNA affects WNT signalling. Importantly, we uncover that these molecular defects are also observed in sporadic ALS MNs, thereby highlighting a common mechanism underlying familial and sporadic ALS.

Discussion and Conclusions:
These findings expose WNT as a driver of motor neuron degeneration in ALS. In FUS-ALS MNs this is, at least in part, driven by FUS-mediated disruption to non-coding RNAs, highlighting the importance of RNA processing in familial and sporadic ALS.

HCB-30: MiRNA expression in motor neurons of the oculomotor nucleus in ALS

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Background:
The oculomotor nucleus responsible for eye movement is typically spared in patients with ALS whereas the spinal cord exhibits substantial loss of motor neurons. Understanding the differences between oculomotor nucleus and spinal cord may reveal pathways that permit resistance to ALS progression. Recent studies implicate RNA dysregulation as a source of motor neuron susceptibility in ALS. miRNAs are small non-coding RNA molecules that control post-transcriptional regulation by interacting with mRNA sequences. Quantitative RT-PCR in ALS versus control samples indicate that specific miRNAs (e.g. miR-9-5p) are downregulated in ALS spinal cord[1]. This data suggests that miRNAs are implicated in ALS.

Objectives:
To determine specific miRNA expression in ALS motor neurons of the oculomotor nucleus and spinal cord and compare to corresponding control tissue.

Methods:
Fluorescent in situ hybridization (FISH) was used to detect miRNA expression in fixed tissue sections of midbrain (oculomotor nucleus), medulla (hypoglossal nucleus) and spinal cord from 15 ALS and 5 control patients (medulla was used as an additional control tissue that is affected by ALS). FISH was performed with DIG-labeled LNA probes to miR-9-5p, miR-182-5p, and miR-124-3p. Probes were then fluorescently labelled using tyramide amplification. Sections were imaged with confocal microscopy and fluorescence intensity in motor neurons was measured using Fiji-ImageJ.

Results:
Preliminary results indicated a trend of increased expression for miR-9-5p, miR-182-5p, and miR-124-3p in ALS oculomotor nucleus motor neurons compared to control oculomotor nucleus. These miRNAs were also upregulated in ALS hypoglossal nucleus. miR-182-5p showed the same expression between ALS and control spinal cord motor neurons, whereas miR-9-5p and miR-124-3p showed a trend of decreased expression in ALS spinal cord.

Discussion:
These results indicate that several miRNA species are upregulated in ALS oculomotor nucleus motor neurons and either unchanged or downregulated in spinal cord motor neurons. The upregulated miRNAs in the oculomotor nucleus could either be a consequence of the protection these motor neurons or direct participants in the protection mechanism. Further studies will elucidate the specific role of these miRNAs in oculomotor function of ALS cases and assess if the increased expression is specific or due to a global increase in miRNA biogenesis. These intriguing results will hopefully shed light on a new pathway for ALS treatment.

References:
[1]Hawley ZCE, Campos-Melo D, Strong MJ. MiR-105 and miR-9 regulate the mRNA stability of neuronal intermediate filaments. Implications for the pathogenesis of amyotrophic lateral sclerosis (ALS). Brain Res. 2019 Mar 1;1706:93-100.

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HCB-31: Mislocalization and aggregation of paraspeckle proteins under cellular stress

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Background:
Paraspeckles are nuclear ribonucleoprotein complexes formed by the interaction of the long non-coding RNA NEAT1 (nuclear enriched abundant transcript 1) and members of the DBHS (Drosophila Behaviour Human Splicing) family of proteins, that are located in the interchromatin space (1). They are involved in the regulation of normal gene expression and, through their association with a number of ALS-associated RNA binding proteins (i.e., FUS, TDP-43), have been implicated in the pathogenesis of ALS (2). Paraspeckle proteins have also been suggested to play a role in the stress response (3).

Objectives:
In this study, we examined the subcellular localization of three main paraspeckle proteins: PSPC1, NONO, and SFPQ and their association with the stress granule (SG) marker (TIA-1) under stress condition in vitro.

Methods:
Immunofluorescence (IF) and double-IF using a polyclonal antibody for PSPC1 and monoclonal antibodies for NONO, SFPQ, and TIA-1 were performed to study their subcellular localization under baseline and osmotic stress (400 mM sorbitol, 4h) conditions in permeabilized HEK293T and undifferentiated SH-SY5Y cells. Cells were plated onto coverslips and following stress were fixed using 4% paraformaldehyde. In the case of PSPC1, cells were allowed to recover by removing the stress for 1 and 2 h and returning the cells to standard media. Subcellular localization was examined using a Leica SP8 MP laser scanning confocal microscope with Leica Confocal Software (LASX) and FIJI/ImageJ software used for quantitative analysis.

Results:
Following stress, PSPC1 redistributed from the nucleus to cytoplasm, where it aggregated into fibrils under stress condition in both HEK293T and SH-SY5Y cells. Recovery experiments demonstrated the reversibility of PSPC1 fibrils. While both NONO and SFPQ formed punctate cytoplasmic aggregates following stress, similar aggregates were also observed within the nucleus. Double-IF experiments exhibited colocalization between selected paraspeckle proteins and TIA-1 under stress. We detected more fibrillar SG subtypes, where TIA-1 colocalized with PSPC1 in both HEK293 and SH-SY5Y cells.

Discussion:
We have observed that in response to osmotic stress that the paraspeckle proteins PSPC1, NONO and SFPQ demonstrate nuclear to cytoplasmic redistribution, and that in the case of PSPC1, form reversible pathological fibrils. We have also observed that following a stress response, paraspeckle proteins are associated with stress granules, as suggested by their colocalization with TIA-1. These observations further support a fundamental alteration in RNA metabolism at the level of the regulation of gene expression and in the cellular response to stress in the pathogenesis of ALS.

References:
1. Hirose T et al. Wiley Interdisciplinary Reviews: RNA. 2019 ;10(6):e1545.
2. An H et al. Non-coding RNA Research. 2018 1;3(4):243-52.
3. Fox AH et al. Paraspeckles. Cold Spring Harbor perspectives in biology. 2010 ;2(7):a000687.

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HCB-32: Mitochondrial dysfunction links mutations in TDP-43 and C9orf72 iPS-derived motor neurons from ALS patients

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Background:
Hexanucleotide expansions in the C9orf72 are the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), accounting for up to 50% of familial ALS cases. While mutations in TARDBP are a rare cause of ALS, the deposition of TDP-43 positive cytoplasmic inclusions remains a common neuropathology for approximately 97% of ALS cases, including C9orf72 cases. Identifying common pathways between C9orf72 and TDP-43 would significantly contribute to our understanding of the disease mechanism.
The aim of this study is to identify if C9orf72 and TDP-43 mutations affect mitochondrial function using iPS-derived MNs from patients and isogenic controls.

Methods:
In this study, we differentiated patient motor neurons derived from induced pluripotent stem cells (iPSCs) carrying hexanucleotide expansions in the C9orf72 gene or mutations in TDP-43 (M337V and I383T). We generated isogenic iPSC lines where the expansions were successfully removed by CRISPR/Cas9 in C9orf72 iPSCs. Seahorse XFe was used to assess mitochondrial respiration and live calcium imaging was used to determine mitochondrial calcium buffering.

Results:
We found both C9orf72 and TDP-43 (M337V and I383T) MNs show reduced mitochondrial basal respiration, maximal respiration, spare respiratory capacity and ATP production when stimulated with 0.5 µM FCCP, indicating dysregulation of the mitochondrial complex IV. When stimulated with 100 µM glutamate during live calcium imaging, we found reduced uptake of calcium from the cytosol in C9orf72 and TDP-43 MNs compared to healthy and isogenic controls. Furthermore, we found an imbalance in the expression of MICU1 and MICU2, the gatekeepers of the mitochondrial calcium uniporter, in both C9orf72 and TDP-43 MNs. The mitochondrial potential was also reduced in C9orf72 MNs, while the TDP-43 M337V and I383T MNs did not show differences when compared to healthy controls.

Conclusions:
This study shows that ALS iPS-derived MNs with mutations in C9orf72 and TDP-43 have deficiencies in essential mitochondrial functions, such as respiration, ATP production and calcium buffering.

HCB-33: Mitophagy dysfunction in PBMCs of sporadic ALS patients

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Background:
The presence of dysfunctional mitochondria is widely accepted as a central hallmark in amyotrophic lateral sclerosis (ALS). Although many questions are still unresolved, mechanistic and genetic evidence strongly support a critical contribution of mitochondria to ALS pathogenesis. Mitochondria alterations can be readily found in tissues from patients and animal models, while no data are reported in peripheral blood mononuclear cells (PBMCs).

Objectives:
Aim of this work is to study mitochondrial dynamism, caspase-dependent apoptosis and mitophagy in sporadic ALS (sALS) PBMCs used as peripheral cellular model of the disease (Cereda et al, 2013; Bordoni et al., 2019).

Methods:
We analyzed mitochondria morphology in PBMCs of 32 sALS patients and 32 healthy controls by both Transmission Electron Microscopy (TEM) and MitoTracker® Red CMXRos. We performed cytofluorimetry analysis to evaluate the percentage of damaged mitochondria by staining with Mitotracker and TMRE. We evaluated proteins involved in mitochondrial dynamism and in mitochondria-dependent apoptosis. We evaluated by WB LC3-II/LC3-I ratio and PINK1 protein level for mitophagy. We also analyzed accumulation of damaged mitochondria by the evaluation of localization of PINK1 and LC3 by immunofluorescence microscopy. Finally, we tested the effect of trehalose, which induces autophagy by damaging lysosomes.

Results:
In patients PBMCs, TEM analysis evidenced the presence of morphologically atypical mitochondria. Moreover, we found an increase in mitochondrial area in sALS PBMCs. We then found that the total number of mitochondria is the same, but in sALS PBMCs the percentage of damaged mitochondria is higher respect to healthy control. We did not find any significant change in proteins involved in mitochondrial dynamism and in caspase-dependent apoptosis We further found the clustering of mitochondria in sALS PBMCs. Thus, we investigated mitophagy pathway, finding significant increase in LC3-II/LC3-I ratio ($p<0.05$) and in PINK1 levels ($p<0.05$) in PBMCs of sALS patients. These data suggested us an accumulation of damaged mitochondria, confirmed by the co-localization of PINK1 and LC3 in PBMCs of sALS patients by immunofluorescence microscopy. Finally, treahlose treatment was demonstrated to resolve mitophagy impairment on sALS PBMCs.

Discussion:
Our data suggest that the presence of morphologically altered mitochondria in PBMCs of sALS patients does not rely on an impairment of mitochondrial dynamism or caspase-dependent apoptosis. On the other hand, we found an involvement of mitophagy pathway. Thus, we hypothesized that this impairment could lead to an accumulation of damaged mitochondria, resulting in an inefficient turnover. Finally, trehalose represents an intriguing approach as potential treatments for ALS.

References:
Cereda, Leoni, Milani, et al. PLoS One. 2013 Oct 14;8(10):e75916; Bordoni, Pansarasa, Dell'Orco, et al. J Clin Med. 2019 May 22;8(5):729.

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HCB-34: Mutations in the low complexity domain of TDP-43 perturb autoregulation in murine brain and human pluripotent stem cells

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Live Poster Session B, December 10, 2020, 5:10 PM - 5:50 PM

Background:
Nearly all cases of ALS and half of FTD cases are characterised by cytoplasmic mislocalisation and aggregation of the DNA/RNA-binding protein TDP-43. Given the near ubiquity of TDP-43 pathology in ALS, we recently created the first TDP-43 knock-in mouse, which harbours a single human-equivalent mutation in murine TDP-43. By avoiding transgenic overexpression we revealed that the Q331K mutation causes a critical perturbation in TDP-43 autoregulation. This results in an increase in TDP-43 expression and changes in gene expression and splicing consistent with a gain of TDP-43 function (1).

Objectives:
To determine if additional mutations in the low complexity domain of TDP-43 perturb autoregulation in vivo and if this mechanism of disease is conserved in human cells.

Methods:
RNA was extracted from the frontal cortex of TDP-43 G348C and M323K knock-in mice. Quantitative PCR was used to assess the level of intron 7 splicing and pA1/pA2 polyadenylation site usage in the 3'UTR of the Tardbp transcript, sensitive measures of TDP-43 autoregulation. CRISPR/Cas9 mutagenesis was used to introduce both the Q331K and M337V disease associated mutations into individual KOLF2-C1 human iPSC lines. RNA extracted from these stem cell lines was subjected to the same quantitative PCR assays for TDP-43 autoregulation. An exogenous TDP-43 autoregulation reporter was integrated into both Q331K and isogenic control iPSC lines. The intron 7 splicing assay was adapted to assay autoregulation of the reporter construct in response to endogenous wild-type and mutant TDP-43.

Results:
TDP-43 M323K mice demonstrated reduced intron 7 splicing, reduced pA2 selection and an increase in Tardbp transcription consistent with a loss of autoregulation. Autoregulatory splicing in TDP-43 G348C mice was similar to wild-type littermates, indicating that this mutation does not perturb autoregulation. Both TDP-43 Q331K and M337V iPSCs demonstrated reduced intron 7 splicing, reduced pA2 selection and elevated TARDBP expression, confirming that these disease associated mutation perturb autoregulation in human stem cells. Splicing of the exogenous TDP-43 autoregulation reporter was perturbed in the TDP-43 Q331K iPSC line.

Discussion:
A variety of TDP-43 low complexity domain mutations, including the human disease associated Q331K and M337V mutations directly perturb autoregulation and result in elevated TARDBP expression. TDP-43 G348C does not perturb autoregulation indicating that a specific subregion of the low complexity domain is key for this regulatory function. The mechanism by which these mutations disrupt TDP-43 homeostasis in murine brain is conserved in human stem cells highlighting the relevance of these findings to human disease.

References:
1. White, M.A., Kim, E., Duffy, A., Adalbert, R., Phillips, B.U., Peters, O.M., Stephenson, J., Yang, S., Massenzio, F., Lin, Z., et al. (2018). TDP-43 gains function due to perturbed autoregulation in a Tardbp knock-in mouse model of ALS-FTD. Nat Neurosci 21, 552-563.

HCB-35: Necrosome-positive granulovacuolar degeneration is associated with TDP-43 pathological lesions in the hippocampus of ALS/FTLD cases

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Aim:
Granulovacuolar degeneration (GVD) in Alzheimer's disease (AD) displays the activated necrosome, which is a protein complex consisting of phosphorylated receptor-interacting protein kinase 1 (pRIPK1), pRIPK3 and phosphorylated mixed lineage kinase domain-like protein (pMLKL). Necrosome-positive GVD was associated with neuron loss in AD [1]. GVD was recently linked to the C9ORF72 mutation in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with transactive response DNA-binding protein 43kDa (TDP-43) pathology (FTLD-TDP) [2]. Therefore, we investigated whether GVD in cases of the ALS/FTLD-TDP spectrum shows a similar involvement of the necrosome as in AD, and whether it correlates with diagnosis, presence of protein aggregates and cell death in ALS/FTLD. In addition, we evaluated whether the pyroptotic cell death pathway was altered in these cases, as it was shown that necroptotic signaling can activate the NLRP3 inflammasome to drive inflammation through pyroptosis [3].

Methods:
We analyzed the presence and distribution of the necrosome as well as the pyroptosis effector Gasdermin D in post-mortem brain and spinal cord of ALS and FTLD-TDP patients (n=30) with and without the C9ORF72 mutation, and controls (n=22). We investigated the association of the necrosome with diagnosis, presence of pathological protein aggregates and neuronal loss.

Results:
Necrosome-positive GVD was primarily observed in hippocampal regions in ALS/FTLD-TDP cases and was associated with hippocampal TDP-43 inclusions as main predictor of the pMLKL-GVD stage, as well as with the Braak stage of neurofibrillary tangle pathology. The central cortex and spinal cord, showing motor neuron loss in ALS, were devoid of any pRIPK1, pRIPK3 or pMLKL accumulation. On the other hand, an increased expression of the pyroptosis executioner Gasdermin D was observed in the central cortex of ALS cases.

Conclusions:
Our findings suggest a role for hippocampal TDP-43 pathology as a contributor to the development of necrosome-positive GVD in ALS/FTLD-TDP. The absence of necroptosis-related proteins in motor neurons in ALS argues against a role for necroptosis in ALS-related neuronal demise. However, the increased presence of Gasdermin D in ALS central cortex suggests that the pyroptotic pathway might be important in ALS pathophysiology.

References:
[1] M. J. Koper et al., Acta Neuropathol., vol. 139, pp. 463–484, 2019.
[2] Y. Riku et al., Acta Neuropathol., vol. 138, pp. 783–793, 2019.
[3] D. Frank and J. E. Vince, Cell Death Differ., vol. 26, pp. 99–114, 2019.

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HCB-36: A comprehensive analysis of SFPQ pathology in large ALS patient cohorts

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
Splicing factor proline and glutamine rich (SFPQ) is a RNA-DNA binding protein with roles in key cellular pathways dysregulated in ALS including DNA transcription and repair, RNA processing and transport and paraspeckle formation. Altered processing of SFPQ RNA, specifically increased retention of intron nine, and nuclear depletion of the protein have recently been reported as pathological features of models of ALS1. Importantly, these pathological features have been demonstrated in models based on multiple genetic subtypes of ALS, suggesting that SFPQ dysregulation may be a common pathological feature of this highly heterogeneous disease. This study presents the first comprehensive analysis of SFPQ pathology at the genetic, transcriptomic and protein level in large ALS patient cohorts.

Methods:
SFPQ was examined at the transcript level using a combination of RNA sequencing (RNA-Seq) and quantitative PCR (RT-qPCR). SFPQ at the protein level was assessed by immunoblotting and immunofluorescent staining. Finally, genetic variation in SFPQ was examined at the genetic level by assessing whole-genome sequencing data from a large cohort of sporadic ALS patients.

Results:
Our analysis identified changes in expression and processing of SFPQ mRNA and aberrant SFPQ protein homeostasis in ALS patient tissue. At the genetic level, analysis of our large sporadic ALS patient cohort identified two novel, and two rare sequence variants in SFPQ not previously reported.

Conclusions:
This study confirms that dysregulation of SFPQ is a feature of the central nervous system of ALS patients at the transcript and protein level. These findings indicate that investigations into the functional consequences of this pathology will provide valuable insight into the biology of the disease. The novel variants identified in SFPQ suggest a potential role for genetic variation of SFPQ in ALS, however analysis of additional patient cohorts and functional analyses is required to determine their pathogenic role as causative mutations or risk factors in ALS.

References:
1. Luisier, R. et al. Intron retention and nuclear loss of SFPQ are molecular hallmarks of ALS. Nat. Commun. 9, 2010 (2018).

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HCB-37: Conditioned Medium from Cells Overexpressing TDP-43 Alters the Metabolome of Recipient Cells

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
TDP-43 pathology has been associated with multiple pathways in ALS, such as metabolic dysfunction found in patients and in in vivo models. Currently, it has been described as a “prion-like” protein, as studies have shown its propagation in cell culture from ALS brain extract or overexpressed TDP-43 in co-culture and conditioned medium (1,2), resulting in cytotoxicity. However, the cellular alterations that are associated with this cytotoxicity require further investigation.

Objectives:
Here, we investigated the effects of conditioned medium from HEK-293T cells overexpressing TDP-43 on cellular morphology, proliferation, death, and metabolism.

Methods:
We overexpressed His-tagged, wild-type TDP-43 (wtTDP-43-6×His) in HEK-293T cells for 72 h. The presence of TDP-43 in the medium was measured by ELISA. Naïve recipient HEK-293T cells were incubated with this conditioned medium for 24 h. Propagation of wtTDP-43-6×His was investigated by Western blot of recpient cell lysates. Changes in cell death, proliferation, morphology, energy metabolism, and the metabolome were investigated in recipient cells.

Results:
Cells overexpressing wtTDP-43-6×His (TDP-43 hereafter) released the protein into the conditioned medium within 72 h ($p = 0.0159$, $N = 3$). The protein was not detected in lysates of naive recipient cells incubated in conditioned medium. Cells in TDP-43-conditioned medium showed increased uptake of propidium iodide ($p = 0.1$, $N = 3$), higher proliferation ($p = 0.0286$, $N = 3$), and decreased structural integrity ($p = 0.127$, $N = 3$). Metabolomics analysis revealed alterations in amino acids, spermine/spermidine ratio, t4-OH-Pro, and glutamate in TDP-43-overexpressing cells. Biogenic amines, glycerophospholipids, sphingomyelins, and acylcarnitine were specifically modified in naive cells in TDP-43-conditioned medium. Essential amino acids, proline, glycine, threonine, asparagine, and serine were modified in both conditions.

Discussion:
These findings suggest the toxicity induced by TDP-43-conditioned medium is associated with changes in the metabolome. The metabolic alterations mentioned above have been associated with ALS (3). We observed a metabolism alteration associated with TDP-43 overexpression and TDP-43 conditioned medium with common and distinct discriminant metabolites, suggesting that the putative mechanisms of toxicity are not completely the same. It will be necessary to show the relationship between these metabolism alterations and cell toxicity and to focus on these alterations, regardless of the specificity to TDP-43. Since we did not observe TDP-43 propagation, the results shed light on the hypothesis that TDP-43 propagation could not be the only threatening factor in the medium surrounding cells.

References:
1. Feiler, M.S. et al. J. Cell Boil. 2015, 211, 897–911.
2. Pokrishevsky, E. et al. Sci. Rep. 2016, 6, 22155.
3. Blasco, H. et al. Expert Rev. Neurother. 2020, 1–13.

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HCB-38: HDAC6 inhibition restores TDP-43 pathology and axonal transport defects in human motor neurons with TARDBP mutations

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TAR DNA binding protein 43 kDa (TDP-43) is the major component of pathological inclusions in most patients with sporadic and familial amyotrophic lateral sclerosis (ALS) and up to 50 % of patients with frontotemporal dementia (FTD). ALS patients suffer from progressive degeneration of motor neurons, while FTLD is characterised by the progressive degeneration of cortical neurons in the frontal and anterior temporal lobes. Moreover, heterozygous missense mutations in the gene encoding TDP-43 are a rare cause of ALS. The aim of this study was to investigate whether mutant TDP-43-ALS iPSC derived motor neurons recapitulate aspects of TDP-43 pathology. We generated and characterized induced pluripotent stem cells (iPSCs) from ALS patients with different TARDBP mutations, as well as from three healthy controls and generated an isogenic control. In addition, we also created two different mCherry tagged cell lines starting from a mutant TDP-43 iPSC line, one with mCherry tagging the wild type TDP-43 and another with mCherry tagging mutant TDP-43. These iPSC lines were differentiated into motor neurons and we observed several changes in TDP-43 behaviour e.g. mislocalization to the cytoplasm, accumulation of insoluble TDP-43, C-terminal fragments and phospho-TDP-43. Furthermore, at functional level a defect in mitochondrial motility was noted in motor neurons with a TARDBP mutations compared to control lines. Pharmacological inhibition of histone deacetylase 6 (HDAC6) restored the observed TDP-43 pathologies and the axonal mitochondrial motility in patient-derived motor neurons linking TDP-43 pathologies with axonal mitochondrial transport defects, suggesting that HDAC6 inhibition may be an interesting target for neurodegenerative disorders linked to TDP-43 pathology.

HCB-39: Novel genetic variants and alterations in WWOX levels cause mitochondrial dysfunction in amyotrophic lateral sclerosis

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Background:
Understanding the underlying pathogenic mechanisms and identifying disease-modifiers capable of altering the course of amyotrophic lateral sclerosis (ALS) are crucial for the development of new therapies. One such candidate is the WW domain-containing oxidoreductase (WWOX), whose role in DNA damage response, oxidative stress, neuronal differentiation, and neurodegeneration is widely reported. Here, we sought to verify whether alterations in WWOX signaling may contribute to ALS pathogenesis.

Methods:
Whole cell homogenates were prepared from a large cohort of post-mortem motor cortex (mCTX) from ALS patients and controls. Genetic data from ALS patients was obtained from the Project MinE data browser. Western blots were used to assess alterations in WWOX levels as well as in the levels of proteins involved in the mitochondrial electron transport chain (mtETC). Interactions between WWOX and mtETC proteins were studied by co-immunoprecipitation (co-IP). Cell viability, mtETC proteins levels, and mitochondrial morphology were assessed in human neuroblastoma SH-SY5Y cells following treatment with wild-type and mutant human recombinant WWOX proteins (rWWOX-WT, rWWOX-STOP261E and rWWOOX-STOP353Q).

Results:
Our results demonstrate a significant decrease in WWOX levels in ALS mCTX. Additionally, genetic analysis revealed several rare genetic variants in WWOX in 4,366 ALS samples from Project MinE that were completely absent in gnomAD. Two of these mutations (WWOX-STOP261E and WWOX-STOP353Q) were found in the short-chain alcohol dehydrogenases (SDR) domain, which is involved in regulating the mtETC. Confirming previous studies, our findings reveal a significant decrease in ATP5A and COXII levels in ALS mCTX. Our results also show that WWOX interacts with ATP5A in ALS. To determine whether the two novel ALS mutations in WWOX are linked to mitochondrial dysfunction, SH-SY5Y cells were treated with rWWOX-STOP261E and rWWOX-STOP353Q. Our results indicate that both rWWOX-STOP261E and rWWOX-STOP353Q induce mitochondrial dysfunction, thus reducing cell viability as measured by an MTT assay. Furthermore, we demonstrate that rWWOX-STOP261E and rWWOX-STOP353Q decrease the levels of several proteins involved in the mtETC. Lastly, treatment with rWWOX-STOP353Q reduced mitochondrial length in SH-SY5Y cells.

Discussion:
Taken together, our findings have begun to unravel the role of WWOX mutations in ALS pathogenesis.

HCB-40: Protein-protein interactome perturbation in C9orf72-Amyotrophic Lateral Sclerosis

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Background:
The most common genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) is a GGGGCC hexanucleotide repeat expansion mutation in the C9orf72 gene. RNA repeats are translated into dipeptide repeat proteins (DPRs) that interact with a vast array of host proteins.

Objectives:
We hypothesized that DPRs could introduce a new degree of connectivity between endogenous proteins as well as compete with existing interactions, hence modifying the topology of the protein-protein interaction network. To test this hypothesis, we conducted an in silico analysis of the human protein-protein interactome perturbation following introduction of poly-GR and poly-PR and their interactions.

Methods:
Published poly-GR and poly-PR interactions1 were introduced into a comprehensive human protein-protein interaction network curated from publicly available databases. Base and perturbed interactomes were partitioned into modules of highly connected proteins using a modified Louvain community detection algorithm.

Results:
The composition of the communities containing TDP-43, the C9ORF72 protein or SQSTM1/p62, three proteins implicated in the pathomechanism of C9orf72-associated ALS, was compared between the base and the perturbed interactomes. Novel interactions introduced by DPRs modify the composition of the TDP-43-containing community and of the C9ORF72-containing community but not the composition of the SQSTM1/p62-containing community. Gene Ontology (GO) term enrichment annotation shows an abundance of terms related to RNA processing in the TDP-43-containing community in the base interactome, which is consistent with the well-established function of TDP-43 in RNA metabolism. By contrast, GO terms in the TDP-43-containing community in the perturbed interactome are enriched in terms related to RNA catabolism. GO term enrichment analysis shows an abundance of terms related to autophagy in the C9ORF72 community in the base interactome, but the highest enriched terms in the C9ORF72-containing community in the perturbed interactome are related to intracellular vesicular transport. Introduction of two nodes with random interactions did not perturb the architecture of the TDP-43, C9ORF72 or SQSTM1/p62 communities from the base interactome, confirming the specificity of the change in the community structure resulting from DPR interactions.

Discussion and conclusions:
These results suggest that DPRs perturb connectivities within the protein-protein interactome. DPR-induced perturbations would promote the function of TDP-43 in RNA destabilisation. Such a possibility is consistent with experimental results demonstrating destabilisation of specific TDP-43 RNA targets in C9orf72 expansion carriers2. Specific components of the TDP-43 community after DPR introduction represent candidate proteins mediating this process. DPR-induced perturbations would also promote the function of C9ORF72 protein in vesicular transport. Network analysis of protein interactome perturbation by DPRs provides a unique platform to get insight into pathways affected in C9orf72-associated ALS.

References:
1. Lee, K.H. et al. (2016). Cell 167: 774-788.
2.Tank, E.M. et al. (2018). Nat. Commun. 9: 2845.

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HCB-41: Role of C9orf72 in AMPAR Trafficking

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Live Poster Session C, December 11, 2020, 12:05 PM - 12:50 PM

Abstract:
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease targeting motor neurons of the brain and spinal cord, causing paralysis and death. Over 50% of ALS patients also develop cognitive impairment caused by degeneration of the frontal and temporal lobes with 15% of these patients fulfilling the diagnostic criteria of frontotemporal dementia (FTD). As such, ALS and FTD are considered the two extremes of a broad neurodegenerative spectrum. The most common genetic cause of ALS/FTD is G4C2 hexanucleotide repeat expansions in C9orf72, a gene of largely unknown function. The repeat expansions in C9orf72 cause haploinsufficiency and reduced C9orf72 protein expression. It is therefore important to fully elucidate C9orf72 function as a means to understanding the pathogenic mechanism causing ALS/FTD. Evidence has indicated that C9orf72 is a DENN domain containing protein involved in molecular trafficking. Directly related to this, our lab has demonstrated that there are increased post-synaptic levels of GluR1 in C9orf72 knockout mice and this coincides with decreased levels of Rab39b. Glutamate excitoxicity has long been considered a pathogenic mechanism in ALS and our findings provide a direct link between C9orf72 mutations and potential deficiencies in AMPAR trafficking. With the aim of establishing the role of C9orf72 in AMPAR endo/exocytosis, we performed immunofluorescence staining on HeLa cells expressing GluR1 tagged with superecliptic (SEP-GluR1) to visualize GluR1 trafficking. Analysis of our preliminary data shows that the expression level of SEP-GluR1 is higher in C9orf72 knockout HeLa cells compared to control cells, in line with our hypothesis that C9orf72 deletion may be associated with elevated level of GluR1. Further works on the mechanisms and functional consequences of elevlated levels of GluR1 are being performed.

HCB-42: SFPQ role in the accumulation of RNA foci and dipeptide repeat proteins from the expanded repeat mutation in C9orf72

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Background:
Paraspeckle core protein SFPQ has been related to ALS/FTD pathology in several studies (1–3). It was recently shown by us that, along with other core paraspeckle proteins, SFPQ interacts with sense RNA foci in formation of paraspeckle-like bodies and reduction in its expression level leads to reduction of sense foci number (4).

Objectives:
Defining role of SFPQ in RNA foci formation and dipeptide protein (DPR) production form C9orf72 mutation.

Methods:
In vitro protein-RNA interaction was checked by RNA pull-down assay using long RNA constructs (48xG4C2, 32xC4G2) in combination with immunoblot. SPFQ overexpression was performed in HEK cells expressing G4C2 or C4G2 repeats. SFPQ knock down was performed on HEK cells and C9orf72 mutation positive fibroblasts by lentiviral transduction of shRNA. Cells lysates were analyzed for DPR expression by dot blots and fixed cells were analyzed for RNA foci expression by RNA fluorescence in situ hybridization and immunofluorescent staining (IF) of proteins.

Results:
SFPQ binding was confirmed for sense RNA, while there was no in vitro interaction with antisense RNA. Overexpression of SFPQ in HEK cells significantly increased foci number relative to control, having bigger impact on sense foci number. DPR production was also significantly increased. Knock down of SFPQ in HEK cells lead to decrease in number of both sense and antisense RNA foci as well as DPR production relative to control. In C9orf72 mutation positive fibroblasts SFPQ KD decreased sense foci number in larger extent than antisense foci number relative to control. There was also significant reduction in all DPR expression levels relative to control.

Discussion:
In this study we show for the first time that core paraspeckle protein SFPQ regulates sense and antisense RNA foci number as well as production of all five DPRs. As the impact on sense RNA foci formation is higher than on antisense RNA foci, later being still significant, we propose two mechanisms of action. Firstly, impact on sense foci formation by direct interaction in paraspeckle-like bodies. Secondly, by acting as transcription factor for extended hexanucleotide repeats regulating RNA level of the repeats in cell and, therefore, RNA foci and DPR levels.

References:
1. S. Ishigaki, Y. Fujioka, Y. Okada et al., Cell Rep. 18 (2017) 1118–1131.
2. S. Thomas-Jinu, P.M. Gordon, T. Fielding et al., Neuron 94 (2017) 322-336.e5.
3. R. Luisier, G.E. Tyzack, C.E. Hall et al., Nat. Commun. 9 (2018) 2010.
4. A. Bajc Česnik, S. Darovic, S. Prpar Mihevc et al., J. Cell Sci. 132 (2019).

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HCB-44: Stem Cell-derived Neurons Provide the Missing Link between ALS Pathology and Motor Neuropathy

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Aggregation of the RNA-binding protein TDP-43 in vulnerable neurons is the diagnostic pathology for most patients with amyotrophic lateral sclerosis (ALS), which is characterized by the selective loss of motor neurons. Furthermore, mutations in the gene TARDBP, which encodes for TDP-43, is a cause of familial ALS. Although it has been proposed that these genetic and pathological perturbations disrupt normal RNA metabolism, the identity of the RNAs regulated by TDP-43 in human neurons remains poorly understood. Here, we used RNA sequencing to identify transcripts whose abundances in purified human stem cell-derived motor neurons (hMNs) were sensitive to reduced TDP-43 levels. We found that transcript levels of Stathmin 2 (STMN2), a regulator of microtubule stability and neurite extension normally highly expressed in motor neurons, were reproducibly and sharply decreased. This reduction was also the case in hMNs differentiated from patient-derived induced pluripotent stem cell lines with pathogenic TDP-43 mutations. STMN2 loss upon altered TDP-43 function was due to altered splicing, which is functionally important, as we demonstrate STMN2 is necessary for normal axonal outgrowth and regeneration. Although hMNs generated in vitro share key molecular and functional properties with bona fide hMNs, the in vivo validation of discoveries from stem cell-based models of ALS is a critical test of their relevance to disease mechanisms. To this end, we used ALS patient spinal cord tissues to provide in vivo evidence corroborating our disease modeling studies that TDP-43 dysregulation alters the expression of STMN2 through altered splicing. We further leveraged this molecular information of altered STMN2 splicing to develop a potential ALS biomarker assay, and we have identified compounds that can correct this splicing defect that could serve as an ALS therapeutic. In conclusion, findings from human stem cell-based models can be used to discover unique aspects of human biology underlying disease pathomechanisms and can illuminate potential therapeutic targets and disease biomarkers.

HCB-45: Stress Granules Regulate Stress-induced Paraspeckle Assembly

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Eukaryotic cells contain a variety of RNA-protein macrocomplexes termed RNP granules. Different types of granules share multiple protein components; however, the crosstalk between spatially separated granules remains unaddressed. Paraspeckles and stress granules (SGs) are prototypical RNP granules localized exclusively in the nucleus and cytoplasm, respectively. Both granules are implicated in human diseases, such as amyotrophic lateral sclerosis. We characterized the composition of affinity-purified paraspeckle-like structures and found a significant overlap between the proteomes of paraspeckles and SGs. We further show that paraspeckle hyperassembly is typical for cells subjected to SG-inducing stresses. Using chemical and genetic disruption of SGs, we demonstrate that formation of microscopically visible SGs is required to trigger and maintain stress-induced paraspeckle assembly. Mechanistically, SGs may sequester negative regulators of paraspeckle formation, such as UBAP2L, alleviating their inhibitory effect on paraspeckles. Our study reveals a novel function for SGs as positive regulators of nuclear RNP granule assembly and suggests a role for disturbed SG-paraspeckle crosstalk in human disease.

HCB-46: Target fibers in ALS are associated with selective autophagy-mediated clearance of denervated fibers

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Background:
The histological feature of skeletal muscles in ALS is the presence of denervated and reinnervated myofibers. Moreover, target/targetoid fibers (TFs) are frequently observed as abnormal structures of myofibers in ALS. The components of TFs have been identified as LC3, desmin, αB-crystallin, dystrophin, filamin C, and Hsp27. However, the biological significance of TFs remains unclear.

Objectives:
The present study aimed to determine the biological significance of the formation of TFs in neurogenic muscular atrophy in ALS.

Methods:
We retrospectively analysed the medical records of 17 consecutive ALS patients who were admitted to our institution from January 2005 to December 2017, and who underwent muscle biopsy to exclude myopathies. We recorded the clinical characteristics in serial ALS patients with and without TFs. We next investigated the histological features of TFs, which involved classifying myofibers with TFs into type I, type IIA/IIB, or type IIC, or categorising myofibers with TFs into grouped atrophic fibers, small angulated fibers, or fiber type grouping. We then performed immunohistochemistry for LC3, p62, beclin-1, and ubiquitin.

Results:
Among 331 patients who were eventually diagnosed with ALS based on Awaji criteria, 17 ALS patients (4 men, 13 women) underwent muscle biopsy. Serum creatine kinase (CK) levels and % forced vital capacity were higher in TF-positive group, compared to TF-negative group. Serum CK levels were higher in the cases with myogenic changes than those without the changes. TFs were frequently observed in myofibers of type I fibers and fiber type grouping, suggesting a reinnervation process. LC3, beclin-1, p62, and ubiquitin were frequently located at the center of the sarcoplasm with TFs. Ubiquitin and p62 were colocalized in the TFs.

Discussion:
A recent study revealed increased signals of ER proteins/chaperones and autophagy markers (LAMP, p62 and LC3) in denervated muscle fibers of ALS, especially at sites of 'targets', indicating partly the dynamic response of muscle fibers to denervation.1 Z disk is subjected to stresses during muscle contraction. For maintenance of Z disk, chaperon-assisted selective autophagy reportedly facilitates the degradation of damaged components, such as filamin, through the coordination of BAG-3 with Hsc70, HspB8, CHIP, and p62.2 Of note, most of these molecules have been shown to be components of TFs. During the denervation-reinnervation process, clearance of Z disk components by chaperon-assisted selective autophagy might be involved in the biology of TFs.

References:
1. Jesse CM, Bushuven E, Tripathi P et al Brain Pathol 2017; 27:781-94.
2. Arndt V, Dick N, Tawo R et al Curr Biol 2010; 20:143-8.

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HCB-47: Tau hyperphosphorylation and mis-localization in amyotrophic lateral sclerosis

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Background:
The exact molecular mechanisms leading to motor neuron loss in amyotrophic lateral sclerosis (ALS) are not yet fully elucidated. Recent studies have described alterations in tau in both sporadic and familial cases of the disease, however, it is unclear if alterations in tau contribute to the pathogenic mechanisms underlying ALS. Here, we sought to verify whether tau is mis-localized and hyperphosphorylated in a larger cohort of ALS post-mortem motor cortex (mCTX).

Methods:
Whole cell homogenates, nuclear, cytoplasmic, and synaptoneurosome (SNs) fractions were obtained from post-mortem mCTX from ALS and controls. Western blots and immunohistochemistry were used to assess alterations in tau and phosphorylated tau (pTau). Electron microscopy was used to assess axonal degeneration in ALS and control mCTX. Co-immunoprecipitation (Co-IP) studies were performed to assess the interactions between proteins, such as the non-receptor tyrosine kinase Fyn and the post-synaptic density protein 95 (PSD95) as well as between Fyn and pTau. SH-SY5Y cells were used to assess pTau levels following treatment with recombinant tau, ALS or control SNs in the absence or presence of a selective tau degrader (QC-01-175). Novel genetic variants in MAPT gene were identified in ALS using the ALS Knowledge Portal (ALSKP) and Project MinE data browser.

Results:
Our findings demonstrated the presence of tau fibrils in both the gray and white matter of ALS mCTX together with an increase in axonal degeneration. Interestingly, hyperphosphorylated tau at S396 and S404 mis-localized to the nucleus and synapses in ALS mCTX, reminiscent of Alzheimer's disease (AD). Increases in pTau-S396 and pTau-S404 were independent of sex, region of onset, and genotype. Similar to AD, Fyn interacted with PSD95 and pTau in ALS mCTX. Importantly, the treatment of SH-SY5Y cells with the selective tau degrader, QC-01-175, reduced increases in pTau-S396 and decreased the interaction between pTau-S396 and Fyn induced by ALS SNs treatment. Lastly, we identified specific genetic variants in MAPT in ALS by assessing ALSKP and Project MinE.

Discussion:
Collectively, our results suggest that tau hyperphosphorylation and mis-localization may play a role in ALS pathogenesis and that targeting hyperphosphorylated tau with the novel degrader QC-01-175 mitigates these effects.

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HCB-48: TDP-43 condensation properties specify its RNA-binding and regulatory repertoire

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Background:
Mutations that cause Motor Neuron Disease (MND) have been shown to affect condensation properties of the RNA binding protein (RBP) TDP-43 that is central to the pathogenesis of MND. Less is known, however, about the role of RBP condensation in the selection of specific endogenous RNA binding sites, and thereby regulation of RNAs.

Objectives:
To address the question of TDP-43's RNA specificity, we created cell lines for inducible expression of TDP-43. We introduced deletions and mutations into the C-terminal intrinsically disordered region (IDR) of TDP-43 that change the condensation properties on a gradient from negative to positive effects both in vitro and in vivo.

Methods:
Using UV crosslinking and immunoprecipitation (iCLIP) and 3' mRNA-Seq, we show that TDP-43 condensation promotes its efficient assembly on a subset of RNAs binding sites that are characterised by unique sequence features, including a highly multivalent arrangement of GU-rich motifs.

Results:
We establish that the conserved region (CR) within the IDR is crucial for efficient condensation of TDP-43, and we evaluate how CR mutations that affect condensation properties impact on TDP-43's RNA binding and regulatory functions. Notably, we find that point mutations in CR have the same gradient of effects at multiple levels: in vitro condensation, the formation and dynamics of TDP-43 granules in cell nuclei, binding to specific RNA sequences across the transcriptome, and regulation of RNA-processing. The primary feature of RNA sequences that conveys condensation sensitivity is multivalency, i.e., the clustering of multiple motifs within a binding site. However, we find that multivalency alone is not sufficient. The motif clusters need to be unusually long (mainly >100 nt), and the motif sequences and density further determine the sensitivity. We perturb condensation with two approaches, either with CR-mutations or with treatment with the aliphatic alcohol 1,6-hexanediol. We find that these two types of perturbation generally change binding of TDP-43 to different classes of long-multivalent sites, which can be distinguished by motif density and sequence properties.

Discussion and conclusions:
Our findings establish that distinct changes in condensation properties of TDP-43 can affect its assembly and function on distinct subsets of RNAs. Thus, we reveal that altered condensation properties of TDP-43 can selectively fine tune its RNA-regulatory networks by modulating its RNA interactions. We speculate that MND associated alteration in condensation would interfere with this proper network regulation.

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HCB-49: TDP-43 expression is increased in the nucleus and cytoplasm of pericytes simultaneously to PDGFRβ reduction following repetitive oxidative stress in primary human post mortem pericytes

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Background:
Tar DNA-binding protein 43 (TDP-43) cytoplasmic aggregates are found in motor neurons and glia of 97% MND post mortem cases (1). Disease onset has been shown to be driven by mutant TDP-43 in motor neurons, however disease progression is thought to be driven by mutant TDP-43 in glial cells. (3). Pericytes are components of the neurovascular unit , they wrap around capillaries to maintain blood flow and help maintain the blood brain barrier through communication with endothelial cells. In MND there is a (~50%) loss of coverage of the pericyte marker platelet-derived growth factor receptor β (PDGFRβ) on capillaries of the Blood Spinal Cord Barrier (BSCB) (4).

Objectives:
To test the effects of repeated oxidative stress on pericytes in relation to PDGRFβ and TDP-43.

Methods:
Primary human brain pericytes were primed with 1 h Sodium arsenite [1 uM] which was then washed off and replaced with fresh media for 24 h, this was repeated depending on the number of primes. Cells were stained for TDP-43, PDGFRβ, importinα, TIA1 and peIF2α. Plates were imaged on the IXM and quantification of staining intensities were carried out using the journal multi-wavelength cell scoring. Graphpad prism was used to carry out a two-way ANOVA.

Results:
Following 1 h sodium arsenite [1 uM] treatment a reduction in nuclear TDP-43 is observed. Following 1 full priming cycle including recovery time an increase of nuclear TDP-43 compared to vehicle is seen. After each priming cycle an incremental increase in nuclear and cytoplasmic TDP-43 is observed up to 3X priming cycles. At 3X priming cycles cell viability is largely decreased. PDGFRβ is incrementally decreased following each priming cycle.

Discussion:
Following oxidative priming cycles pericytes have low levels of PDGFRβ and increased levels of TDP-43. A mis-localisation of TDP-43 to the cytoplasm is seen after 3 priming cycles. This provides an interesting model where we see pericytes with low levels of PDGFRβ which can be related to the loss of PDGFRβ seen in post mortem MND tissue.

References:
1. Neumann et al., 2006; Science, 314, 130-3.
2. Arai et al., 2006; Biochem Biophys Res Commun, 351, 602-11.
3. Ditsworth et al., 2017; cta Neuropathol, 133, 907-922.
4. Winkler et al., 2013; Acta Neuropathol, 125, 111-20.

HCB-50: The development of iPSC-derived nociceptors for an alternate approach towards studying ALS

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Pain is defined as an unpleasant sensory and emotional experience that occurs in response to noxious stimuli, tissue damage or trauma. While not a primary consequence of ALS, the development of pain as a symptom has detrimental effects on the quality of life experienced by patients. Various types of pain have been reported in approximately 80% of patients diagnosed with ALS, however, despite this there has been little research into the pathomechanistic properties and preventative measures against pain in ALS. Pain with neuropathic features such as spontaneous and evoked symptoms can be described as primary forms of pain and are not commonly seen throughout the pathology of ALS. In contrast, many ALS patients develop pain via secondary mechanisms which arise through changes in connective tissue and joints that are driven by muscle atrophy and weakness as a consequence of motor neuron degeneration. The presence of musculoskeletal pain in ALS patients suggests the activation spinal nociceptors, the afferent neurons responsible for detecting painful and noxious stimuli. The notion that nociceptors may retain their integrity, evidenced by their functional activity, denotes the possibility that they may be resistant to the pathological mechanisms driving the degeneration of motor neurons. Gaining insight into why these nociceptors appear to be resistant to ALS pathology may help understand why motor neurons are particularly susceptible. Furthermore, the utilization of this unusual approach to study ALS pathology may guide the development of strategies to prevent disease pathogenesis and enhance motor neuron survival. Human pluripotent stem cells provide a unique platform to generate human nociceptors that can be investigated in vitro. Using pre-existing protocols and knowledge of developmental biology we have developed a novel small molecule protocol to derive functionally active nociceptors from human PSC's. We found that following neuralization through dual-SMAD inhibition using two well-known inhibitors, SB-431542 and LDN-198189, treatment of neuralized cultures using the Wnt agonist CHIR-99021, retinoic acid and the FGF-inhibitor PD-173074 resulted in the production of a high proportion of neuronal cells expressing a variety of markers indicative of a nociceptive specification including: BRN3A, ISL-1, VGLUT2 and TRK/A. The use of quantitative PCR to assess gene expression confirms results obtained via quantification of immunostained cultures. Furthermore, calcium imaging using FURA2-AM revealed that cultured cells were responsive to known nociceptive stimuli such as Capsaicin and Bradykinin. Using transcriptomics, we plan to investigate transcriptional differences arising in diseased motor neurons vs diseased nociceptors to establish any aberrations in homeostatic mechanisms or functional pathways that could be major contributors to the pathology of ALS.

HCB-51: Transcripts encoding the central stress granule protein G3BP1 are stabilized by TDP-43: potential relevance to ALS/FTD

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TDP-43 nuclear depletion and concurrent cytoplasmic accumulation in vulnerable neurons is a hallmark feature of progressive neurodegenerative proteinopathies such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Cellular stress signalling and stress granule dynamics are now recognized to play a role in ALS/FTD pathogenesis. Defective stress granule assembly is associated with increased cellular vulnerability and death. G3BP1 (Ras-GAP SH3-domain-binding protein 1) is a critical stress granule assembly factor. Here, we define that TDP-43 stabilizes G3BP1 transcripts via direct binding of a highly conserved cis regulatory element within the 3'UTR. Moreover, we show in vitro and in vivo that nuclear TDP-43 depletion is sufficient to reduce G3BP1 protein levels. Finally, we establish that G3BP1 transcripts are reduced in ALS/FTD patient neurons bearing TDP-43 cytoplasmic inclusions/nuclear depletion. Thus, our data suggest that, in ALS/FTD, there is a compromised stress granule response in disease-affected neurons due to impaired G3BP1 mRNA stability caused by TDP-43 nuclear depletion. These data implicate TDP-43 and G3BP1 loss of function as contributors to disease.

HCB-52: Unraveling the role of TDP43 proteinopathy and its therapeutic modulation in C9orf72-related and sporadic ALS in vitro models

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Background:
The cytoplasmic accumulation and aggregate formation of hyper-phosphorylated and ubiquitinated TDP43 is the pathological signature of the great majority of Amyotrophic lateral sclerosis/Frontotemporal dementia (ALS/FTD), including C9Orf72-related familial forms (fALS) and sporadic forms (sALS). Impairment in nucleocytoplasmic transport (NCT) and TDP43 cytoplasmic mislocalization have been extensively reported in ALS, including C9-ALS.

Objectives:
To validate TDP43 cytoplasmic mislocalization in cell lines derived from C9-ALS patients, compared to controls, sALS and other fALS and to appraise the effect of its therapeutic modulation. To test the role of TDP43 mitochondrial localization in causing neuronal toxicity in ALS. To assess the expression of the nucleoporin POM121 in sALS and fALS lines and its involvement in TDP43 cellular localization.

Methods:
ALS patient and control fibroblasts obtained from skin biopsy were reprogrammed to induced-pluripotent stem cells (iPSCs) and differentiated into motor neurons (MNs). Assessment of TDP43 cellular localization was performed by immunofluorescence in all lines. ALS fibroblasts received a 24-hour treatment with Verdinexor, a selective inhibitor of nuclear export (SINE). Immunofluorescence, RT-PCR, Western blot for nuclear, cytoplasmic and mitochondrial fractions, as well as biochemical analysis were performed on fibroblasts of ALS patients and compared to controls.

Results:
TDP43 cytoplasmic mislocalization was found in fibroblasts, but not in iPSCs and MNs, of patients with sALS, and to a large extent with fALS due to TDP43 and C9orf72 mutations. Preliminary results showed that C9-ALS fibroblasts may have an increased mitochondrial content of TDP43, higher number of mitochondria compared to controls, morphological mitochondrial alterations, as well as impairment in mitochondrial activity. Verdinexor was able to revert TDP43 cytoplasmic mislocalization in ALS fibroblasts and to ameliorate mitochondrial damage. POM121 levels were reduced in C9-ALS compared to controls.

Discussion:
Decrease in nucleocytoplasmic ratio was identified in ALS fibroblasts and not in unstressed iPSCs and MNs, suggesting that TDP43 cytoplasmic mislocalization may be a relevant but age-related hallmark in ALS. Impairment in NCT is of paramount importance in ALS pathogenesis, especially in C9-ALS, and could at least in part responsible for mitochondrial dysfunction in C9-ALS. Therapeutic SINE-mediated TDP43 relocalization and nucleoporin level restoration may be promising therapeutic strategies in ALS.

HCB-53: Y526 phosphorylation modulates aggregation dynamics of FUS

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Background:
Abnormal cytoplasmic accumulation of Fused in Sarcoma (FUS) in neurons defines subtypes of amyotrophic lateral sclerosis (ALS) and fronto-temporal lobar degeneration (FTLD), both accompanied by progressive neuron loss. But the underlying mechanisms leading to FUS mislocalization/aggregation in both diseases appear different. FUS is a nuclear protein that possesses non-classical PY-type nuclear localization signal (NLS) at its C-terminus[1]. In ALS but not in FTLD, the mutations in the NLS of FUS are responsible for its impaired nuclear transport mediated by receptor transportin 1 (TNPO1) which is affected also by post-translational modifications of FUS[2]. We reported on posttranslational modification - the phosphorylation of C-terminal tyrosine at position 526 in NLS of FUS, which abolishes FUS interaction with TNPO1 and impairs its nuclear import[3].

Objective:
As kinases were shown to phosphorylate Y526, we aimed to elaborate on cell compartment specific phosphorylation of Y526 in mutated, truncated and full length FUS, by kinases of the Src-family.

Methods:
Custom antibodies against phosphorylated FUS Y526 were developed and their specificity confirmed by siRNA silencing, immunoprecipitation, phosphatase assays and in knock-out mice. Further, the activities of Src-famaily of kinases (c-Src, c-Fyn, c-Abl) of were analysed in cells, and cell models for kinase overexpression and Y526 FUS phosphorylation analyzed in vitro. The in vivo co-localization of active kinase and phosphorylated Y526 FUS signals were examined by immunohistochemistry on frozen sections of mouse brain.

Results:
We showed that mutated FUS, C-terminal fragments of FUS and full length FUS, when phosphorylated at Y526 form morphologically distinct aggregates with kinase-dependent localization within the cells. Moreover, in mice brain slices the phosphorylated FUS signal demonstrated cell type specific.

Discussion:
This suggest on kinase-type mediated distinct FUS inclusion formation in cells, possibly associated with variable progression of neurodegenerative diseases. The limited cytoplasmic phosphorylation of Y526 may nonetheless serve to ensure for the small level of FUS to remain in the cytoplasm possibly for dendritic mRNA transport.

References:
1. Lee BJ, Cansizoglu AE, Su KE et al. Cells 2006; 126: 543–558.
2. Hofweber M, Hutten S, Bourgeois B et al. Cell 2018; 173 (3): 706-719.
3. Darovic S, Prpar Mihevc S, Župunski V, et al. J Cell Sci 2015; 128: 4151-4159

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