COVID 19: Decoding the mechanisms of neurotoxicity and inflammation.

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The Problem

Over 700 million people have been infected with SARS-CoV-2 (COVID-19), and ~10% of individuals suffer with symptoms 3+ months beyond acute infection. The neurological effects of COVID-19 share many similarities to neurodegenerative diseases. Our team has demonstrated that the ORF6 protein in the SARS-CoV-2 virus can form amyloid structure (Figure 1). The ORF6 peptides are toxic and impair mitochondrial function in a neuroblastoma cell line (SH-SY5Y) (1,2).

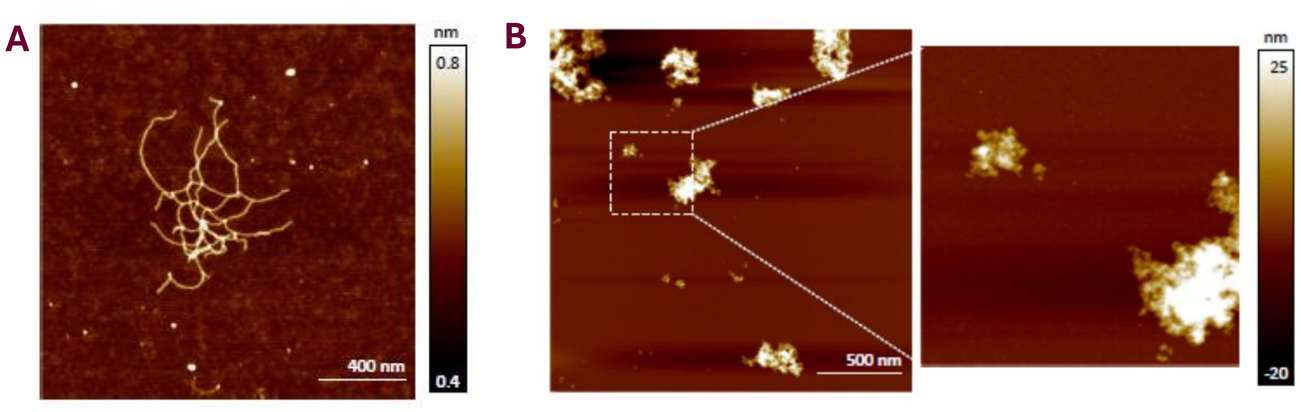

Figure 1 Atomic force microscopy image of tORF6 amyloid fibres (A) and ORF6 forming amyloid like structure (B) (3).

Hypothesis and Aim

- ORF6 protein forms toxic amyloids which aggregate and accumulate in the brain and leads to the neurological symptoms in both acute COVID-19 and Long COVID.
- To determine if ORF6 causes toxicity to cell populations within the brain and whether this is via NLRP3 inflammasome mediated inflammation. To answer this aim different brain cell types will be utilised, including iBMDM cells as a proxy for microglia cells.

Methodology

Cells: iBMDM and iBMDM NLRP3 -/- cells were treated 24 hours post seeding with protein.
Protein: Addition of full length ORF6 protein (61 amino acids) or truncated ORF6 (40 amino acids) at 55 µM (low toxicity) or 110 µM (high toxicity) concentration.

Assays

1. Mitochondria function: Oxygen consumption rate (OCR) was measured using Seahorse Analyzer at 48 hours with different pharmacological agents to inhibit components of respiration.
2. Cell Viability: At 48 hours MTT was added to cells with the reaction stopped and colour change measured at 490 nm, indicating cell viability.

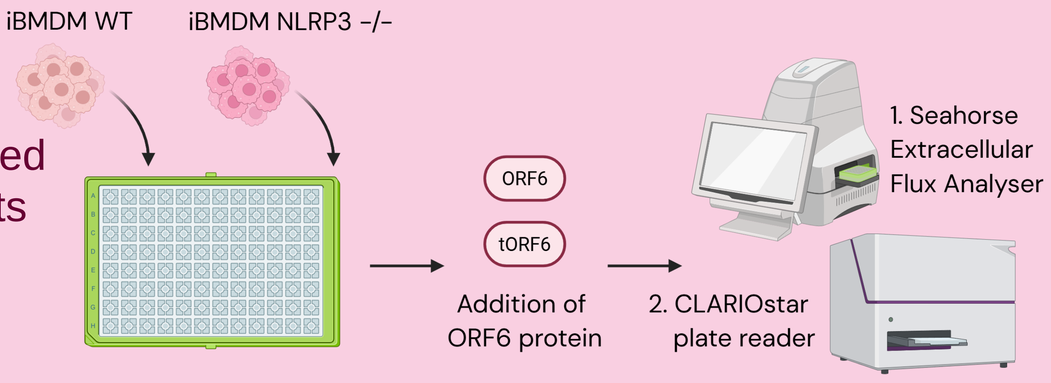

Figure 2 Workflow of cell seeding, protein addition and assays utilised.

ORF6 protein reduces basal respiration in iBMDM and NLRP3 -/- cells

Basal respiration is significantly lower in iBMDM cells, with all components reduced. Although showing a downward trend NLRP3 -/- does not reach significance, indicating NLRP3 is not essential for ORF6 mediated effect on mitochondria respiration.

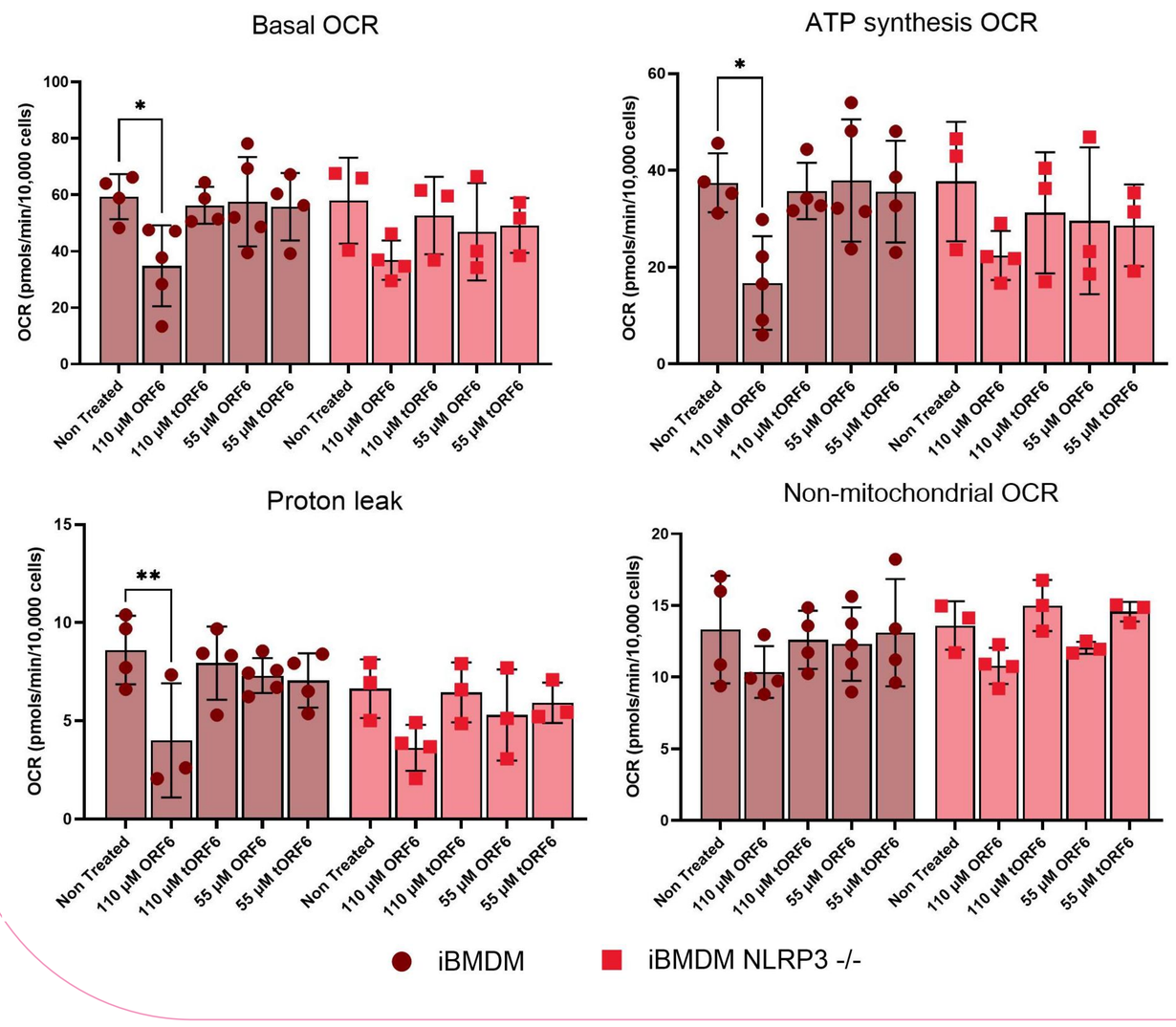

Figure 3 Values were normalised to cell number to account for differences in cell seeding. Statistical analysis was performed by two-way ANOVA with Dunnett test performed to correct for multiple comparisons, with n=3. Significance: * = P<0.05 and ** P=0.01.

ORF6 protein is not toxic to iBMDM cells at 55µM and 110µM concentrations

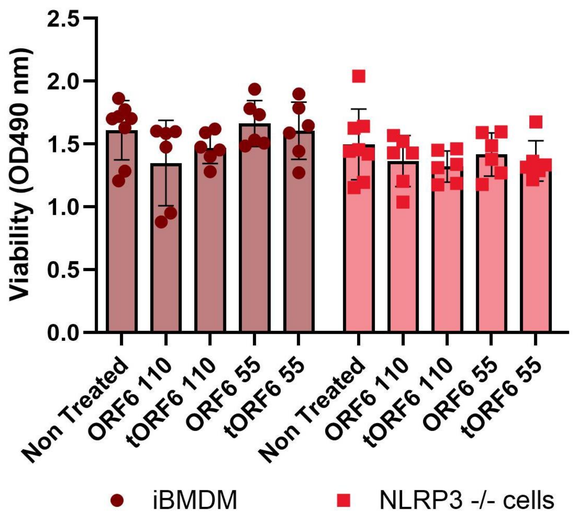

Figure 4 Cell viability as a read out of optical density (OD) 490nm. Results are not significant. Values were normalised to cell number to account for differences in cell seeding. Statistical analysis was performed by two-way ANOVA with Sidak test performed to correct for multiple comparisons, with n=3.

What's next?

Assess metabolic pathways, cell viability and pro-inflammatory cytokine production in the following models:

- Addition of supernatant from ORF6 SH-SY5Y cells to iBMDMs
- Co-culture of iBMDM's with SH-SY5Y stably expressing ORF6
- Addition of ORF6 protein to organoids containing neuronal cells, astrocytes and microglia

1. Charnley M, Islam S, Bindra GK et al. Neurotoxic amyloidogenic peptides in the proteome of SARS-COV2: potential implications for neurological symptoms in COVID-19. Nat Commun. 2022;13(1):3387.
2. Sanislav O, Tetaj R, Metali, Ratcliffe J, et al. Cell invasive amyloid assemblies from SARS-CoV-2 peptides can form multiple polymorphs with varying neurotoxicity. Nanoscale. 2024;16(42):19814-27
3. Preliminary data provided by Nick Reynolds, RMIT university and ACMD.

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